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### MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA-LACTAMASE AMONG CLINICAL MULTIDRUG RESISTANT *ESCHERICHIA COLI* IN TWO HOSPITALS OF NIAMEY, NIGER

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#### ABSTRACT

**Objective:** The aim of this study was to identify the multiple ESBL genes in Multidrug-resistant (MDR) *Escherichia coli* isolated in various biological samples in two hospitals of Niamey.

**Methodology:** A total of 195 multidrug-resistant *Escherichia coli* were included in the study. These isolates were tested using polymerase chain reaction (PCR) for detection of the presence of *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>OXA-1</sub> beta-lactamase genes.

**Results:** A total of 27.7% of *Escherichia coli* isolates were ESBL producing strains. Globally, the *bla*<sub>TEM</sub> gene was the most prevalent (70.3%) followed by *bla*<sub>CTX-M</sub> (43.1%), *bla*<sub>OXA-1</sub> (31.8%) and *bla*<sub>SHV</sub> (4.1%) genes. The four genes type of ESBL were founded simultaneously only in stool samples. Furthermore, none *bla*<sub>SHV</sub> gene was found in other samples type.

**Conclusion:** This study showed the presence of various ESBL genes among clinical MDR *Escherichia coli*. That is why a rational use of antibiotic and appropriate methods of screening ESBL genes in routine laboratories in Niger is needed to control the ESBL genes dissemination.

**Keywords:** MDR *Escherichia coli*, ESBL, *bla* genes, PCR, Niamey, Niger.

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### CARACTERISATION MOLECULAIRE DES BETALACTAMASES A SPECTRE ETENDU CHEZ LES SOUCHES DE *ESCHERICHIA COLI* MULTI RESISTANTES DANS DEUX HOPITAUX DE NIAMEY, AU NIGER

Fody<sup>1,2\*</sup>, A. M. , Bagré<sup>1,3</sup>, T. S. , Traoré<sup>1</sup>, A. K., Yacouba <sup>4</sup>, A., Dembelé<sup>1</sup>, R., Boubou<sup>2</sup>, L. , Inoussa<sup>2</sup>, A., Sidikou,<sup>5</sup> R., Traoré,<sup>1</sup> A. S. , Gassama-Sow,<sup>3</sup> A. and Barro,<sup>1</sup> N.

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#### RESUME

**Objectifs:** Le but de cette étude était d'identifier les multiples gènes de BLSE chez les souches de *Escherichia coli* multi résistantes isolées de différents types d'échantillons biologiques dans deux hôpitaux de Niamey.

**Méthodologie :** Un total de 195 *Escherichia coli* multi résistants a été inclus dans l'étude. Ces isolats ont été testés par la réaction de polymérase en chaîne (PCR) pour détecter la présence des gènes *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> et *bla*<sub>OXA-1</sub>.

**Résultats :** Au total, 27,7% des isolats de *Escherichia coli* multi-résistants étaient des souches productrices de BLSE. Globalement le gène *bla*<sub>TEM</sub> (70,3%) était le plus détecté suivi des autres gènes *bla*<sub>CTX-M</sub> (43,1%), *bla*<sub>OXA-1</sub> (31,8%) et *bla*<sub>SHV</sub> (4,1%). Notons que seul dans les échantillons de selles quatre types de gènes de BLSE ont été trouvés simultanément. Par ailleurs notons qu'aucun gène de type *bla*<sub>SHV</sub> n'a été trouvé dans les autres types d'échantillons.

**Conclusion :** Cette étude avait montré la présence de divers gènes de BLSE chez les souches cliniques de *Escherichia coli*. C'est pourquoi une utilisation rationnelle des antibiotiques et des méthodes appropriées de dépistage des gènes de BLSE dans les laboratoires sont nécessaires afin de contrôler la diffusion des gènes de BLSE.

**Mots clés :** *Escherichia coli* multi résistantes, BLSE, gènes *bla*, PCR, Niamey, Niger

## INTRODUCTION

Resistance to beta-lactam antibiotics is an increasing problem and beta-lactamase production in Gram-negative bacteria, is one of the most common mechanisms of drug resistance. Beta-Lactamases, very diversified due to their continuous mutation, belong to the Ambler classes A (1) and are usually plasmid-encoded that also harbor resistant genes to other antimicrobial classes with resulting multidrug-resistant isolates (2, 3). These multidrug resistant (MDR) Gram negative bacilli belonging to the *Enterobacteriaceae* family are increasingly responsible for hospital infections (bacteremia urinary tract and intra-abdominal infection) in many countries (4, 5). The MDR extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* have become a concern in medical bacteriology regarding antimicrobial treatment and infection control in hospitals (6). Indeed, these enzymes (ESBL) have the capacity to hydrolyze extended-spectrum cephalosporins and monobactam antibiotics, but are inhibited by clavulanic acid (7, 8). The emergence of ESBL-producing isolates has important clinical and therapeutic implications. High prevalence of ESBL-producing *Enterobacteriaceae* has been reported in the literature for clinical samples from a variety of infection sites (9). In the past decade, there has been a significant increase in the prevalence of resistance to extended spectrum cephalosporin in *Escherichia coli* (10). From the clinical and epidemiological side, the ESBL-producing *Escherichia coli* represent a significant therapeutic challenge as they are resistant to all beta-lactam antibiotics currently available and other antibiotic families alternative (fluoroquinolones, cotrimoxazole, aminosides or tetracyclines), to except cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem and ertapenem) (11, 12). Different types of ESBLs have been found in different

countries. There are currently three main ESBL (TEM, SHV and CTX-M) types, which are the most widespread and clinically relevant (13). The TEM and SHV types were first reported from *Klebsiella pneumoniae* in Western Europe. In the late 1990s, the prevalence of TEM and SHV had decreased, while that of CTX-M, especially associated to species *Escherichia coli* increased (10). The CTX-M type beta-lactamases represent a rapidly emerging group worldwide, which have been found in *Enterobacteriaceae*, particularly in *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Salmonella typhimurium* (14). Genotypic tests have the potential to accurately identify different genes encoding the ESBLs (15). Several reports have described the prevalence of ESBLs genes in the Middle East North Africa region and most of the Gulf Cooperation Countries (16). So the detection of specific genes by PCR and sequencing are commonly used for final confirmation of ESBL producers. The association of ESBLs and the presence of TEM, SHV, OXA and CTX-M-type enzymes have been investigated in many studies (17). However, there are insufficient scientific data on the ESBLs gene characterization available from Niger.

To our knowledge, no data are so far available on ESBL-producing *Escherichia coli* clinical isolates in Niger. The aim of this study was to screen the presence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub> and *bla*<sub>CTX-M</sub> genes among clinical MDR *Escherichia coli* isolated from various biological samples in Niamey.

## MATERIALS AND METHODS

### Samples collection

The present prospective study was conducted from March 2014 to June 2016 in the medical biology laboratory of "hôpital national de Niamey" and "hôpital national Lamordé" in Niamey, Niger. The isolates were obtained from various samples. Multidrug resistant *Escherichia coli* isolates, were isolated from stool (n=49), urinary tract infection

(n=134), pus (n=7), blood (n=4), and vaginal swabs (n=1) samples.

**Bacterial identification and antibiotic susceptibility testing**

Isolation, identification, antimicrobial susceptibility testing of isolates and phenotypic characterization of ESBL were described in our previous study (18). All isolates included in this study were multidrug resistant.

**Preparation of DNA**

Total DNA was extracted as previously described by Steward (19) using boiling process. Samples were cultured onto Bromo-Cresol Pourpre (BCP) agar and incubated at 37°C for 24 hours. For DNA extraction, two loopful of each strain were homogenized into 250 µl of sterile water. The mixture was boiled for 10 min and centrifuged for 10 min. After 5 min at room

temperature, the supernatant was collected and used for the PCR reactions.

**Detection of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub> and *bla*<sub>CTX-M</sub> genes.**

The beta-lactamase genes *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA-1</sub>, and *bla*<sub>CTX-M</sub> were detected by PCR using specific primers (Table 1). PCR mixtures were prepared by using 2.5 µl of template DNA, 4.0 µl of Master Mix PCR (Solis BioDyne, Estonia), 0.5 µl of Forward primer, 0.5 µl of Reverse primer in a final volume of 20 µl. Amplification conditions were 94°C for 5 min following 35 cycles of 94°C for 30 Sec, 55°C for 60 Sec, and 72°C for 60 Sec with a final extension at 72°C for 10 min. PCR amplified fragments were separated by agarose gel (1% w/v) electrophoresis in 1 x TAE buffer and visualized under UV light.

**TABLE 1: BETA-LACTAMASE GENES PRIMERS**

Genes	Primers	Sequences (5'-3')	Amplicon size (bp)
<i>bla</i> <sub>TEM</sub>	TEM-F	ATAAAATTCCTGAAGACGAAA	1080
	TEM-R	GACAGTTACCAATGCTTAATCA	
<i>bla</i> <sub>SHV</sub>	SHV-F	TTATCTCCCTGTTAGCCACC	650
	SHV-R	GATTGCTGATTTCCGCTCGG	
<i>bla</i> <sub>OXA-1</sub>	OXA-1-F	ATGAAAAACACAATACATATC	890
	OXA-1-R	AATTTAGTGTGTTAGAATGG	
<i>bla</i> <sub>CTX-M</sub>	CTX-M-F	GTTACAATGTGTGAGAAGCAG	593
	CTX-M-R	CCGTTTCCGCTATTACAAAC	

**Ethical considerations**

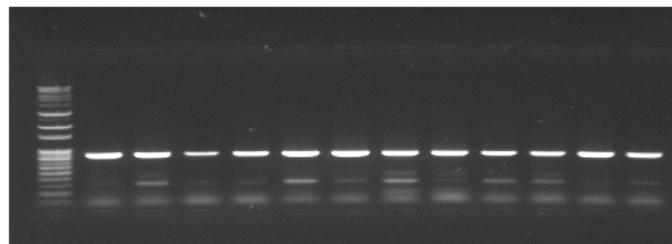
All biological specimens were collected as part of the routine clinical management of patients. The study was approved by the medical establishment committee of “hôpital national de Niamey” and “hôpital national Lamordé” in Niamey and permission to conduct the study was obtained from the hospital authorities of Niger.

**Statistical analysis**

Data analysis was carried out using Microsoft Excel 2013 and Med Cal version 11.0.1.0. P < 0.05 was considered to be statistically significant.

**RESULTS**

A total of 195 MDR *Escherichia coli* were included in this study. Among these isolates, 27.7% (54/195) were positive for the phenotypic character of extended spectrum beta-lactamases. Molecular analysis had shown the presence of various beta-lactamase genes. The genes identified by PCR method using specific primers i.e TEM, SHV CTX-M and OXA-1 on agarose gel were shown in Figures 1 and 2.



**FIGURE 1: PCR AMPLIFICATION OF *E. COLI* *bla*<sub>TEM</sub> GENES**

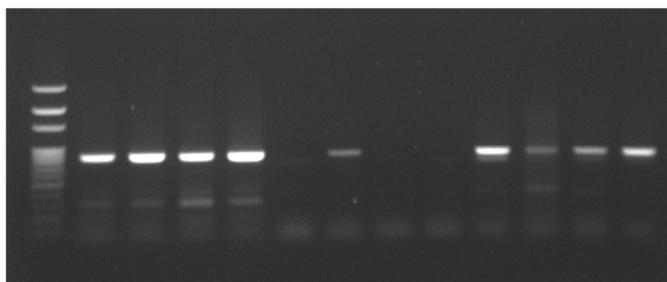


FIGURE 2: PCR AMPLIFICATION OF *E. COLI* *bla*<sub>CTX-M</sub> GENES

Forty nine (49) MDR *Escherichia coli* isolates from stool samples were tested for ESBL resistant genes. Overall, *bla*<sub>TEM</sub> gene was the most prevalent (93.9%) followed by *bla*<sub>OXA-1</sub> (71.4%), *bla*<sub>CTX-M</sub> (65.3%) and *bla*<sub>SHV</sub> (16.3%) ( $P < 0.0001$ ) (Table 2). All the fourteen (14) ESBL isolates positive by phenotypic method, were

positive for the *bla*<sub>TEM</sub> gene (100%), 85.7% for the *bla*<sub>CTX-M</sub> gene, 85.7% for the *bla*<sub>OXA-1</sub> gene and 21.4% for *bla*<sub>SHV</sub> gene ( $P < 0.0001$ ) as indicated in Table 3. Globally, *bla*<sub>SHV</sub> gene was only detected in stool samples.

TABLE 2: DISTRIBUTION OF BETA-LACTAMASE GENES FROM MDR *E. COLI*

ESBL genes	Clinical source				
	Stools N=49	Urine N=134	Pus N=7	Blood N=4	Vaginal swabs N=1
<i>bla</i> <sub>TEM</sub> n (%)	46 (93.9)	82 (61.2)	4 (57.1)	4 (100)	1 (100)
<i>bla</i> <sub>SHV</sub> n (%)	8 (16.3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> <sub>OXA-1</sub> n (%)	35 (71.4)	20 (14.9)	5 (71.4)	2 (50)	0 (0)
<i>bla</i> <sub>CTX-M</sub> n (%)	32 (65.3)	45 (33.6)	5 (71.4)	2 (50)	0 (0)

About the 134 MDR *Escherichia coli* isolated from urine samples, *bla*<sub>TEM</sub> gene was the most prevalent (61.2%), followed by *bla*<sub>CTX-M</sub> (33.6%) and *bla*<sub>OXA-1</sub> (14.9%) respectively (Table 2). Out of the thirty-seven (37) ESBL isolates positive by phenotypic method, 27 (73.0%) were positive for *bla*<sub>TEM</sub> gene, 13 (35.1%) for *bla*<sub>CTX-M</sub> gene, and 3 (8.1%) for *bla*<sub>OXA-1</sub> gene ( $P < 0.0001$ ) (Table 3). None of the isolates was positive for *bla*<sub>SHV</sub> genes.

The study of seven (7) MDR *Escherichia coli* isolated from pus samples, showed the presence of *bla*<sub>CTX-M</sub> gene, *bla*<sub>OXA-1</sub> gene and *bla*<sub>TEM</sub> gene with a prevalence of 71.4%, 71.4% and 57.1% respectively. The *bla*<sub>SHV</sub> gene was not found in any of the isolates (Table 2). There was no significant difference between the expressing of these genes in pus samples ( $P = 0.3735$ ).

Only the *bla*<sub>CTX-M</sub> gene (50%) and *bla*<sub>OXA-1</sub> gene (50%) were found in *Escherichia coli* isolates positive for ESBL by phenotypic method (Table 3).

In the four (4) MDR *Escherichia coli* isolated from blood culture samples, *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub> and *bla*<sub>CTX-M</sub> genes were found in 100%, 50%, and 50% respectively (Table 2). However, *bla*<sub>TEM</sub> (100%), *bla*<sub>OXA-1</sub> (100%) and *bla*<sub>CTX-M</sub> (100%) were found in *Escherichia coli* isolates producing ESBL by phenotypic method (Table 3).

Concerning vaginal swabs, only *bla*<sub>TEM</sub> gene was found in *Escherichia coli* isolates as indicated in Table 2 and Table 3.

TABLE 3: DISTRIBUTION OF ESBL GENES FROM MDR *E COLI* POSITIVE TO ESBL

ESBL genes	Clinical source									
	Stool		Urine		Pus		Blood		Vaginal swabs	
	ESBL + N=14	ESBL - N=35	ESBL + N=37	ESBL - N=97	ESBL+ N=2	ESBL - N=5	ESBL+ N=1	ESBL - N=3	ESBL+ N=0	ESBL - N=1
<i>bla</i> <sub>TEM</sub> n (%)	14 (100)	32 (91.4)	27 (73.0)	55 (56.7)	0 (0)	4 (80)	1 (100)	3 (100)	0 (0)	1 (100)
<i>bla</i> <sub>SHV</sub> n (%)	3 (21.4)	5 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> <sub>OXA-1</sub> n (%)	12 (85.7)	23 (65.7)	3 (8.1)	17 (17.5)	1 (50)	4 (80)	1 (100)	1 (33.3)	0 (0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> n (%)	12 (85.7)	20 (57.1)	13 (35.1)	32 (33.0)	1 (50)	4 (80)	1 (100)	1 (33.3)	0 (0)	0 (0)

In this study, there was co-existence of ESBL genes in some *Escherichia coli* isolates. The co-existence of *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes was found in stool, blood, pus and urine samples in 63.3%, 50%, 42.9%, 22.4% (P = 0.0002) respectively, while *bla*<sub>CTX-M</sub> gene and *bla*<sub>OXA-1</sub> gene were detected in 57.1%, 50.0%, 71.4%, and 4.5% respectively. The co-existence of three ESBL genes belonging to group *bla*<sub>CTX-M</sub> + *bla*<sub>TEM</sub> + *bla*<sub>OXA-1</sub> genes was observed in 57.1%, 50%, 28.6% and 3%

respectively from stool, blood, pus and urine samples (P < 0.0001). However, other multiple co-detection of ESBL genes belonging to different groups: *bla*<sub>CTX-M</sub> + *bla*<sub>SHV</sub> (14.3%), *bla*<sub>CTX-M</sub> + *bla*<sub>TEM</sub> + *bla*<sub>SHV</sub> (12.2%), *bla*<sub>CTX-M</sub> + *bla*<sub>SHV</sub> + *bla*<sub>OXA-1</sub> (14.3%) were observed in stool samples. A combination of four genes (12.2%), *bla*<sub>CTX-M</sub> + *bla*<sub>TEM</sub> + *bla*<sub>SHV</sub> + *bla*<sub>OXA-1</sub> genes was found only in *Escherichia coli* isolated from stool samples (Table 4).

TABLE 4: DISTRIBUTION OF VARIOUS ESBL GENES COMBINATIONS ACCORDING TO CLINICAL SAMPLES

ESBL genes combination	Clinical sources				
	Stool N=49	Urine N=134	Pus N= 7	Blood N=4	Vaginal swabs N=1
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>TEM</sub> n (%)	31 (63.3)	30 (22.4)	3 (42.9)	2 (50.0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>SHV</sub> n (%)	7 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>OXA-1</sub> n (%)	28 (57.1)	6 (4.5)	5 (71.4)	2 (50.0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>TEM</sub> + <i>bla</i> <sub>SHV</sub> n (%)	6 (12.2)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>TEM</sub> + <i>bla</i> <sub>OXA-1</sub> n (%)	28 (57.1)	4 (3.0)	2 (28.6)	2 (50.0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>SHV</sub> + <i>bla</i> <sub>OXA-1</sub> n (%)	7 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>TEM</sub> + <i>bla</i> <sub>SHV</sub> + <i>bla</i> <sub>OXA-1</sub> n (%)	6 (12.2)	0 (0)	0 (0)	0 (0)	0 (0)

DISCUSSION

Over the last decades, many studies have demonstrated the presence of ESBL resistance genes in bacteria isolated from patients during various infections (20 -22). This study reported the results of molecular characterization of ESBL resistance genes in MDR *Escherichia coli* strains isolated in two hospitals of Niamey, Niger.

The study of *Escherichia coli* producing ESBL (14 isolates) isolated from stool samples, had shown that the most frequent ESBL gene was *bla*<sub>TEM</sub> followed by *bla*<sub>CTX-M</sub>, *bla*<sub>OXA-1</sub> and *bla*<sub>SHV</sub> genes. However, previous studies from Qatar reported a higher prevalence of *bla*<sub>CTX-M</sub> gene (66.1%), *bla*<sub>SHV</sub> (53.2%) and *bla*<sub>TEM</sub> (40.4%) (9). In another study, the predominant ESBL genes, were *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> and

*bla*<sub>SHV</sub> (23). Other studies in clinical isolates showed that *E. coli* harbored *bla*<sub>SHV</sub> gene (88.9%), *bla*<sub>OXA</sub> (44.4%) and *bla*<sub>CTX-M</sub> genes (44.4), and *bla*<sub>TEM</sub> gene (22.2%) (24). These results were contradictory to our results. The difference would be due to the sampling from different hospitals and from different geographic locations, whereas in our study, samples were collected only from two hospital setting.

This study revealed that, out of the 37 *E coli* producing ESBL isolated from urine samples, the percentage of *bla*<sub>TEM</sub> was 73.0% followed by *bla*<sub>CTX-M</sub> (35.1%) and *bla*<sub>OXA-1</sub> (8.1%). Karimian et al. in Iran, reported similar data: *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes were observed in 71.2% and 37.3% of *E. coli* isolates, respectively (25). In our study, none of the isolate harbored *bla*<sub>SHV</sub> gene. A similar finding was reported

from Thai studies. (26). But our results contradicted those reported in some studies conducted by Egyptian and Indian authors, in which *bla*<sub>SHV</sub> gene was detected among urinary *E. coli* isolates (27, 28). Also, in East Africa, ESBL genes, *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>OXA</sub> were found frequently (23). Thus, this required a better understanding of the ESBL genes in order to give useful data about their epidemiology. In this study, only 4 MDR *Escherichia coli* isolated from blood culture were tested for ESBL genes. Thus, the co-existence of *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub> and *bla*<sub>CTX-M</sub> was only seen in one strain producing EBSL. Our results correlated with other results reported in some African's countries which showed a significantly lower proportion of ESBL in blood cultures than in other specimens (29). The previous finding was contradicted with the finding of a Burkina Faso study which had shown the highest proportion of ESBL genes in blood samples (30). Whereas, none of ESBL-producing isolates carried *bla*<sub>SHV</sub> or *bla*<sub>CTX-M</sub> genes as reported in Ghana (31). This discrepancy can be explained by differences in the antimicrobials use between countries.

The present study reported the multiple co-existence of ESBL genes. Thus, 63.3% of isolates from stool samples harbored two ESBL genes belonging to groups of *bla*<sub>CTX-M</sub> + *bla*<sub>TEM</sub>, followed by blood samples (50%), pus samples (42.9%) and urine samples (22.4%). The greatest frequency of beta lactamase gene in *E. coli* samples was related to TEM/CTX-M in previous study as reported in Iran (32). The co-existence of *bla*<sub>TEM</sub> + *bla*<sub>CTX-M</sub> genes and *bla*<sub>SHV</sub> + *bla*<sub>CTX-M</sub> genes in two strains were reported in India (33).

This study revealed that the co-existence of three (3) ESBL genes (*bla*<sub>CTX-M</sub> + *bla*<sub>TEM</sub> + *bla*<sub>OXA-1</sub>) was observed in 57.1%, 50%, 28.6% and 3% of the *Escherichia coli* isolates respectively in stool, blood,

## REFERENCES

1. Ambler, R. P., Coulson, A. F., Frère, J. M., Ghuysen, J. M., Joris, B., Forsman, M., Levesque, R. C., Tiraby, G., and Waley, S. G. A standard numbering scheme for the class A betalactamases. *Biochem J.* 1991; 276: 269–270
2. Dallenne, C., Da Costa, A., Decré, D., Favier, C. and Arlet, G. Development of a set of multiplex PCR assays for the detection of genes encoding important b-lactamases in *Enterobacteriaceae*. *J Antimicrob Chemother.* 2010; 65: 490–495
3. Rakotonirina, H. C., Garin, B., Randrianirina, F. Richard, V. Talarmin, A. and Arlet, G. Molecular characterization of multidrug-resistant Extended-spectrum β-lactamase-producing *Enterobacteriaceae*

pus and urine samples. This result was also found in another study conducted in Bangladesh (24). These results indicated that ESBL-producing *Escherichia coli* frequently harbored more than one beta-lactamase genes.

Overall, only isolates from stool samples harbored simultaneously four (4) ESBL genes (*bla*<sub>CTX-M</sub> + *bla*<sub>TEM</sub> + *bla*<sub>SHV</sub> + *bla*<sub>OXA-1</sub>). According to a previous study in Lebanon, 15.9% of *Escherichia coli* harbored the four ESBL genes (34). The genetic diversity of *bla* genes in isolates, suggested that resistance genes can easily move from one specie to another with the possibility of easy interspecies transfer.

This study was conducted in two hospitals of Niamey; it may reflect the local antibiotic resistance patterns and this may be a limitation.

## CONCLUSION

This first study in Niger showed a high incidence of ESBL resistance genes among clinical *Escherichia coli* isolates from two laboratories in two hospitals (Hôpital National de Niamey and Hôpital National Lamordé) of Niamey. Many isolates harboring more than one ESBL resistance genes, were also detected. Therefore, immediate implementation and recommendations for antimicrobial management and infection control measures were necessary to prevent the spread of these genes across the whole country.

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isolated in Antananarivo, Madagascar. *BMC Microbiol.* 2013; 13: 85

4. Sun, H. Y., Chen, S. Y., Chang, S. C., Pan, S. C., Su, C. P., and Chen, Y. C. Community-Onset *Escherichia coli* and *Klebsiella pneumoniae* bacteremia: Influence of Health Care Exposure on Antimicrobial Susceptibility. *Diagn Microbiol Infect Dis.* 2006; 55: 135-141
5. Canton, R., Novais, A., Valverde, A., Machado, E., Peixe, L. Baquero, F. and Coque, T. M Prevalence and Spread of Extended-Spectrum Betalactamase-Producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect.* 2008; 14: 144-153
6. Drieux, L. Brossier, F. Sougakoff, W. and Jarlier, V. Phenotypic detection of extended-spectrum

- Beta-lactamase production in *Enterobacteriaceae*: review and bench guide. *Clin Microbiol Infect.* 2008; 14: 90-103
7. Paterson, D. L. and Bonomo, R. A. Extended-Spectrum  $\beta$ -Lactamases: A Clinical Update. *Clin Microbiol Rev.* 2005; 18: 657-686
  8. Lina, T. T., Khajanchi, B. K., Azmi, I. J., Islam, M. A., Akter, B. M. M., Banik, A., Alim, R., Navarro, A., Perez, G., Cravioto, A., Talukder, K. A. Phenotypic and Molecular Characterization of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Bangladesh. *PLoS ONE.* 2014; 9: e108735
  9. Ahmed, M. A. S., Bansal, D., Acharya, A., Elmi, A. A., Hamid, J. M., Ahmed, A. M. S., Chandra, P., Ibrahim, E., Sultan, A. A., Doiphode, S., Bilal, N. E. and Deshmukh, A. Antimicrobial susceptibility and molecular epidemiology of extended-spectrum betalactamase producing *Enterobacteriaceae* from intensive care units at Hamad Medical Corporation, Qatar. *Antimicrob Res Infect Control.* 2016 ; 5 : 4
  10. Canton, R., Coque, T. M. The CTX-M beta lactamase pandemic. *Curr Opin Microbiol.* 2006 ; 9 : 466-75
  11. Grall, N., Andremont, A., Armand-Lefèvre, L. Résistance aux carbapénèmes: vers une nouvelle impasse? *J Anti-infect.* 2011; 13: 87-102
  12. El bouamri, M. C., Arsalane, L., Zerouali, K., Katfy, K., El kamouni, Y., Zouhair, S. Molecular characterization of extended spectrum-lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa. *Afr J Uro.* 2015; 21: 161-166
  13. Giamarellou, H. Multidrug resistance in Gram-negative bacteria that produce extended-spectrum b-lactamases (ESBLs). *Clin Microbiol Infect.* 2005; 11: 1-16
  14. Liu, W., Chen, L., Li, H., Duan, H., Zhang, Y., Liang, X., Li1, X., Zou1, M., Xu, L. and Hawkey, P. M. Novel CTX-M beta lactamase genotype distribution and spread into multiple species of *Enterobacteriaceae* in Changsha, Southern China. *J Antimicrob Chemother.* 2009; 63: 895-900
  15. Polsfuss, S., Bloemberg, G. V., Giger, J., Meyer, V., Böttger, E. C., Hombach, M. Evaluation of a diagnostic flow-chart for detection and confirmation of extended spectrum b-lactamases (ESBL) in *Enterobacteriaceae*. *Clin Microbiol Infect.* 2012; 18: 1194-2004
  16. Zowawi, H. M., Balkhy, H. H., Walsh, T. R., Paterson, D. L. Betactamase Production in Key Gram-Negative Pathogen Isolates from the Arabian Peninsula. *Clin Microbiol Rev.* 2013; 26: 361-80
  17. Mugnaioli, C., Luzzaro, F., De Luca, F., Brigante, G., Perilli, M., Amicosante, G., Stefani, S., Toniolo, A., and Rossolini, G. M. CTX-M type extended-spectrum beta-lactamases in Italy: molecular epidemiology of an emerging countrywide problem. *Antimicrob Agents Chemother.* 2006; 50: 2700-2706
  18. Fody, A. M., Boubou, L., Moussa, A., Bawa, H. I., Konaté, A., Yaou, C., Zongo, C., Salaou, C., Daouda, A., Sidikou, R., Traoré, A. S. and Barro, N. Phenotypic detection of extended spectrum betalactamase in multidrug-resistant *Escherichia coli* from clinical isolates in Niamey, Niger. *Afr. J. Microbiol. Res.* 2017; 11: 712-717
  19. Steward, C. D., Rasheed, J. K., Hubert, S. K., Biddle, J. W., Raney, P. M., Anderson, G. J., Williams, P. P., Britain, K. L., Oliver, A., McGowan, J. E., and Tenover, F. C. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum beta-lactamase detection methods. *J Clin Microbiol.* 2001; 39: 2864-2872
  20. Hoban, D. J., Lascols, C., Nicolle, L. E., Badal, R., Bouchillon, S., Hackel, M., Hawser, S. Antimicrobial susceptibility of *Enterobacteriaceae*, including molecular characterization of extended-spectrum beta-lactamase-producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study. *Diagn Microbiol Infect Dis.* 2012; 74: 62-7
  21. Sharma, M., Pathak, S., Srivastava, P. Prevalence and antibiogram of extended spectrum  $\beta$ -lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella spp.* *J Clin Diagn Res.* 2013; 7: 2173-7
  22. Tansarli, G. S., Poulidakos, P., Kapaskelis, A., Falagas, M. E. Proportion of extended-spectrum  $\beta$ -lactamase (ESBL)-producing isolates among *Enterobacteriaceae* in Africa: evaluation of the evidence-systematic review. *J Antimicrob Chemother.* 2014; 69: 1177-84
  23. Sonda, T., Kumburu, H., Zwetselaar, M. V., Alifrangis, M., Lund, O., Kibiki, G. and Aarestrup, F. M. Meta-analysis of proportion estimates of

Extended-Spectrum-Beta-Lactamase producing *Enterobacteriaceae* in East Africa hospitals. *Antimicrob Res Infect Control*. 2016; 5: 18

24. Islam, S. S., Malek, Md. A., Haque, A. K. M. F., Talukder, K. A., Akhter, M. Z. Beta Lactamase Genes of Extended Spectrum Beta Lactamase Producing *Escherichia coli* from Anorectal Sepsis Cases in Bangladesh. *Bangladesh J Microbiol*. 2013; 30: 23-29

25. Karimian, M., Rostamzad, A., Shoaee, P. Extended spectrum b-lactamase-producing strains of *Escherichia coli* in hospitalized children in Isfahan, Iran. *Avicenna J Clin Microbiol Infect*. 2015; 2: e27096.

26. Udomsantisuk, N. P., Nunthapisud, T., Tirawatanapong, and Dansuputra, M. Molecular characterization of extended spectrum beta-lactamase among clinical isolates *Escherichia coli* and *Klebsiella pneumoniae*. *J. Med. Assoc. Thai*. 2011; 94: 1504-1512.

27. Salah, M., Azab, M., Halaby, H., Hanora, A. Mutations in  $\beta$ -lactamases detected in multidrug resistant gram negative bacteria isolated from community acquired urinary tract infections in Assiut, Egypt. *Afr. J. Microbiol. Res*. 2016; 10: 1938-1943

28. Nisha, K. V., Veena, S. A., Rathika, S. D., Vijaya, S. M., Avinash, S. K. Antimicrobial susceptibility, risk factors and prevalence of *bla* cefotaximase, temoneira, and sulfhydryl variable genes among *Escherichia coli* in community-acquired pediatric urinary tract infection. *J Lab Physicians*. 2017; 9: 156-62.

29. Sangare, S. A., Maiga, A. I., Guindo, I., Maiga, A., Camara, N., Savadogo, S., Diallo, S., Bougoudogo, F., Armand-Lefevre, L., Andremont, A., Maiga, I. I. Prevalence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* isolated from blood cultures in Africa. *Med Mal Infect*. 2015; 45: 374-82

30. Ouedraogo, A. S., Sanou, M., Kissou, A., Sanou, S., Solaré, H., Kaboré, F., Poda, A., Aberkane, S., Bouzinbi, N., Sano, I., Nacro, B., Sangaré, L., Carrière, C., Decré, D., Ouégraogo, R., Jean-Pierre, H. and Godreuil, S. High prevalence of extended-spectrum  $\beta$ -lactamase producing *Enterobacteriaceae* among clinical isolates in Burkina Faso. *BMC Infect Dis*. 2016; 16: 326

31. Mensah, D. O., Nkrumah, N. O., Bonney, E. Y., Mensah, E. O., Danso, K. T., Osei, Y. D. and Sackey, S. T. Genetic characterization of TEM-type ESBL-associated antibacterial resistance in *Enterobacteriaceae* in a tertiary hospital in Ghana. *Ann Clin Microbiol Antimicrob*. 2016; 15: 29

32. Tayh, G., Sallem, R., Yahia, H. B., Gharsa, H., Klibi, N., Boudabous, A., Slama, K. B. First report of extended-spectrum beta-lactamases among clinical isolates of *Escherichia coli* in Gaza Strip, Palestine. *J Glob Antimicrob Res*. 2016; 6: 17-21

33. Krishnamurthy, V., Vijaykumar, G. S., Kumar, S. M., Prashanth, H. V., Prakash, R., Nagaraj, E. R. Phenotypic and Genotypic Methods for Detection of Extended Spectrum  $\beta$  Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Ventilator Associated Pneumonia. *J Clin Diagn Res*. 2013; 7: 1975-1978

34. Daoud, Z., Sokhn, E. S., Masri, K., Cheaito, K., Ahmad, N. H., Matar, G. M. and Doron, S. *Escherichia coli* isolated from urinary tract infections of Lebanese patients between 2005 and 2012: epidemiology and profiles of resistance. *Front Med*. 2015; 2: 11

## ORIGINAL ARTICLE

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### IN VITRO ANTIMICROBIAL ACTIVITY OF FERMENTED SPICES AND *CAPSICUM FRUTESCENS* AGAINST MULTI DRUG RESISTANCE CLINICAL ISOLATE AND STANDARD REFERENCE BACTERIA

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#### ABSTRACT

**Introduction:** Food preservation is required to maintain for a long period of time. Traditional organic food preservative, "Datta" is spice mainly made up of Chili Peppers which frequently used in southern and western part of Ethiopia. Datta can be consumed almost with every kind of foods and it is believed as appetizer and antimicrobial agent against food borne pathogen. This study aimed to assess *in vitro* antimicrobial activity of fermented condiment and *Capsicum frutescens* against multi drug resistance clinical isolate and standard reference bacteria.

**Method:** Datta samples collected from different level hotels and *Capsicum frutescens* (Chili peppers) were extracted in different solvents. Agar well diffusion assay was used to determine antimicrobial activity and minimum inhibitory concentration (MIC) and minimum bactericidal concentration was determined by tube dilution method. One way analysis of variance was used in comparison of the finding.

**Results:** Extracted fermented condiment (Datta) sample and Chili Pepper showed antimicrobial activities against multidrug resistant clinical isolate and standard reference bacteria in well diffusion assay. Datta extract showed MIC ranged from 25 mg/L to 66.7 mg/L and MBC ranged from 25 mg/L to 100 mg/L. The Datta and Chili pepper extracts showed high antimicrobial activities against standard *Staphylococcus aureus*. The water based extract of Datta sample were exhibited significantly low antimicrobial activities ( $P=0.000$ ) as compared to the other extraction solvents.

**Conclusion:** Water was weak extractor of active compounds having antimicrobial activities. Reference *S. aureus* was more susceptible organism while ATCC *Salmonella enteritidis* and clinical isolated multi-drug resistant *E. coli* less susceptible. The traditional use of fermented condiment for food preservation by the local people is supported by this study.

**Key words:** Antimicrobial activity; Chili Pepper extract; Fermented condiment; Minimum bactericidal concentration; Minimum inhibition contraction

### ACTIVITÉ ANTIMICROBIENNE EN VITRO D'ÉPICES FERMENTÉES ET DE FRUITS DE *CAPSICUM FRUTESCENS* POUR LA RÉSISTANCE AUX MÉDICAMENTS ISOLATE CLINIQUE ET BACTÉRIES DE RÉFÉRENCE STANDARD

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## ABSTRAIT

**Introduction:** La conservation des aliments est nécessaire pour maintenir pendant une longue période de temps. Conservateur de nourriture organique et traditionnel, "Datta" est l'épice composée principalement de Chili Peppers qui fréquemment utilise dans le sud et l'ouest de l'Éthiopie. Datta peut être consommé presque avec toutes sortes d'aliments et on le croit comme un apéritif et un agent antimicrobien contre l'agent pathogène alimentaire. Cette étude visait à évaluer l'activité antimicrobienne *in vitro* du condiment fermenté et *Capsicum frutescens* contre l'isolement clinique de résistance aux médicaments multiples et les bactéries de référence standard.

**Méthode:** Les échantillons de Datta prélevés dans des hôtels de différents niveaux et *Capsicum frutescens* (Chili Peppers) ont été extraits dans différents solvants. Un dosage de diffusion de puits a été utilisé pour déterminer l'activité antimicrobienne et la concentration inhibitrice minimale (MIC) et la concentration bactéricide minimale a été déterminée par la méthode de dilution du tube. Une analyse à sens unique de la variante a été utilisée en comparaison de la découverte.

**Résultats:** L'échantillon extrait de condiments fermentés (Datta) et Chili Pepper ont montré des activités antimicrobiennes contre l'isolement clinique résistant aux médicaments multiples et les bactéries de référence standard dans le dosage par diffusion de puits. L'extrait de Datta a montré que le MIC variait de 25 mg/L à 66,7 mg/L et le MBC variait de 25 mg/L à 100 mg/L. Les extraits de poisson de Datta et de Chili ont montré des activités antimicrobiennes élevées contre *Staphylococcus aureus* standard. L'extrait à base d'eau de l'échantillon de Datta a montré des activités antimicrobiennes significativement faibles ( $P = 0,000$ ) par rapport aux autres solvants d'extraction.

**Conclusion:** L'eau était un extracteur faible de composés actifs ayant des activités antimicrobiennes. Référence *S. aureus* était un organisme plus susceptible tandis que ATCC *Salmonella enteritidis* et *E. coli*. *E. coli* résistant aux médicaments multiples isolés était moins susceptible. L'utilisation traditionnelle du condiment fermenté pour la conservation des aliments par les populations locales est soutenue par cette étude.

**Mots clés:** Activité antimicrobienne; Extrait de poivre de piment; Condiment fermenté; Concentration bactéricide minimum; Contraction minimale d'inhibition

## INTRODUCTION

Since ancient time, peoples have been using spices to prevent off-odor, off-flavors and spoilage of foods [1]. Besides flavoring food, currently spices have become an integral part of daily requirements such as for food preservation, cosmetics, medicinal preparation, bakery goods, perfumery, and various other products [2]. The preparation of these spices vary from place to place. One of the well known and the most common spice that is used in most type of food in Ethiopia is known as "Datta" in southern part of the country and "Kotchkocha" in western part in Afan Oromo language. Datta is not heat processed and the main ingredient of this spice is puffed Chili Pepper whereas other ingredients such as Garlic, Ginger, Coriander, and Basil are added in limited amount. All these ingredients crushed together by simple grinding stone until it become semisolid. The Datta acquires original precursor chili pepper color, it became green if it is made of fresh green chili pepper or become red in color if red chili peppers are used [3, 4].

Some foods are not easily contaminated with microorganisms. This may be due to food intrinsic antimicrobial agent of raw materials that have inhibitory effect on growth of

microorganisms. Many secondary metabolites of plants are antibiotic, protecting the plants against different microorganisms [5, 6]. Therefore, herbs are now more focused than ever because they have the capability of producing important metabolites which used as medicine or as precursor for many pharmacological products. Without specific knowledge of their cellular action or mechanisms phytochemicals have been considered possible drugs for times.

Chili peppers are also used worldwide in foods for their pungent flavor, aroma, and to prolong food spoilage. It is one of spice which considered as preservative as well as appetizer by the users. Every country in the world has its own way in making different types of foods and spice. Ethiopia is a country with different ethnic groups with different culture, preparation of spices varies in different part of the country. This study is conducted on Datta which is the most dominant and widely used spice product of pepper known in southern and western part of Ethiopia [3, 4].

Different study showed as Chili Pepper which is the major precursor of Datta has antimicrobial activities on different microorganisms [7, 8, 9]. The Capsaicin present in Chili Pepper is has

antimicrobial activities in addition to other secondary metabolites. Little is known about antimicrobial activities of Datta and scientific evidence is required to confirm its food preservative characteristic of the spice. Searching of effective and safe food preservatives has non debatable benefits now day. There are new concerns about food safety and preservatives due to increasing occurrences of new food-borne disease outbreaks caused by drug resistant pathogenic microorganisms. This advances considerable challenges, mainly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobials to inhibit growth of spoilage microorganisms [10]. These chemical preservatives may have so many side effects as compared to organic preservatives that has a little effect on food flavors are always needed. Spices Chili Peppers may be used as organic food preservative because people are using this condiment during eating delayed food. Currently, there is growing interest in using natural preservatives compounds as alternatives to synthetic compounds for food preservation [11]. In this study, the antimicrobial activity of Chili Pepper spices is going to be investigated as an alternative to antibiotics and food preservative option. The aim of this study is to assess *in vitro* antimicrobial activity of fermented condiment and chili pepper against multi drug resistance clinical isolate and standard reference bacteria.

## MATERIALS AND METHODS

### Study design, setting and period

Experimental study design was carried out to assess antimicrobial activity Chili Pepper extract and Arba Minch town Datta collected from high, medium and low level Hotels. The study was conducted in Arba Minch town. The source of Datta's Chili Peppers for Arba Minch town is agriculture land of highland areas surrounding the town and the source of Datta samples were Hotels in the town with different level. Arba Minch town is located 500 kms south of Addis Ababa in southern nation nationality region of Ethiopia situated in the great African rift valley in elevation of 1285 meters above sea level with average temperature of about 29.7°C and the average annual rain fall of 900mm.

### Sample collection

To obtain representative sample, Datta samples for assessing antimicrobial activities against ATCC and MDR clinical bacteria isolates were

collected from hotels having different level. The Datta samples were collected using sterile and leak proof containers and transported to the microbiology laboratory using icebox, and kept in refrigerator during analysis periods. During collection, semi-structured questionnaire and observation check list were used to characterize the Datta samples.

Fresh chili peppers (*Capsicum frutescens*) which used as ingredient for Datta preparation were collected and characterized by local experts. Then the collected peppers were transported to laboratory in aseptic manner. The species name of collected the chili pepper is determined by the help of Taxonomist. Finally collected sample were extracted according to method discussed below.

### Experimental organisms

The screening for antibacterial activities of Datta and Chili pepper crude extracts were carried out by using three bacterial pure cultures: *Staphylococcus aureus* (ATCC® 25923™), *Salmonella enterica subsp. enterica*(ATCC® 13311™) and *Klebsiellapneumoniae* (ATCC® 700603™) and three multi drug resistance clinical bacteria isolates particularly Methicillin resistant *Staphylococcus aureus*(MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*. The organisms were regularly sub-cultures on Nutrient agar slant until screening for antimicrobial activity.

### Datta and Chili Pepper extraction

The collected Datta sample in each hotel was separated into two sterile containers. The first container was used for microbiological characterization of the sample while the second container was for antimicrobial activities. For antimicrobial analysis, 10% w/v proportion was used to extract in four different extraction solvents (Distilled water, Acetone, Ethanol, and Methanol). Then dissolved solution of each sample was kept in orbital shaker for 24 hrs at room temperature. Finally the solution was filtered by Whatman No. 1 filter paper and stored in deep freeze at negative 20°C for further use of antimicrobial analysis [6].

Chili Pepper extraction was performed in six different solvents: Distilled water, Acetone, Ethanol, Methanol, Ethyl acetate, and Chloroform. The collected fresh chili pepper was washed twice in running water and once in distilled. Then it was crushed in disinfected

Morton and pestle. Then 7.5gm of crushed chili pepper will be dissolved in 75ml each solvents to obtain 10% w/v. Then the dissolved solutions were kept on orbital shaker for 24hr. Then the extract was filtered with sterile Whatman No. 1 filter paper and stored in deep freeze at -20°C for further use of antimicrobial analysis.

#### **Agar well diffusion assay of Datta sample and chili pepper extracts**

Datta sample and Chili pepper crude extract was obtained aforementioned methods was evaluated for antimicrobial activity by agar well diffusion assay. Muller Hilton agar (Oxoid Limited, CM0337) is used to perform antimicrobial activity of the extracts. Then agar well diffusion assay was carried out according to method described in clinical and laboratory standards institute [12]. Diffusion well of approximately 6mm diameter was prepared by a sterile micro pipette tip. A 0.5 McFarland standards diluted suspension of each test microorganisms was evenly inoculated on Muller Hilton agar. Then 50µl of the extracts from each sample of Datta and Chili Pepper extract was carefully filled in the well. Additional wells were prepared and filled with each solvent used as negative control. Then the inoculated agar plates were incubated at 37°C for 24 hours. All the tests were conducted in triplicate and the average of the three measurements was used to present the results.

#### **Determination of minimum inhibitory concentration of Datta sample and chili pepper extracts**

Minimum inhibitory concentration (MIC) of Datta sample and chili pepper extract was determined by tube dilution method. The extracted Datta and Chili Pepper were diluted from 100mg/L to 12.5mg/L in nutrient broth (Oxoid). To each test tube with extract, a loop full of  $10^6$  bacteria suspension per ml fresh nutrient broth inoculated in each tube. Then the culture tubes will be incubated at 37°C for 24 hours. After the period of incubation the tubes are checked for turbidity that indicates the growth of bacteria. Then the lowest concentration of Datta sample and pepper extract inhibits growth of the test organisms was considered as the MIC for the respective organisms. To confirm the inhibition, then a loop full of the incubated inoculum was sub-cultured on nutrient agar [5].

#### **Determination of minimum bactericidal concentration of Datta sample and chili pepper extracts**

Minimum bactericidal concentration (MBC) of Datta samples and Chili Pepper extract was determined by tube dilution method followed by confirmation on agar plates. The MIC and the two consecutive preceding concentrations were used to determine MBC. The three concentrations of Datta and Chili Pepper extract were adjusted in Nutrient broth and inoculated with loop full of test organisms. Then the inoculum was incubated at 37°C for 24 hours. After the period of incubation the loop full of the well mixed inoculum from nutrient broth was transferred to nutrient agar and incubated at 37°C for 24 hours. Then the plates were examined for visible colony and the MBC is the lowest concentration that demonstrates no growth of colony.

#### **Statistical Analysis**

Collected data was entered to excel and exported to SPSS version 20.0 for further analysis. Descriptive statistics such as means and standard deviations were calculated. One way ANOVA followed by Tukey's test was used to compare extracts and the difference in the susceptibility of the test microorganisms. The 95% level of confidence ( $P$ -value  $\leq 0.05$ ) was considered as statistically significant.

## **RESULTS**

### **Characteristic of collected Datta sample**

A total of nine Datta samples were collected in different level hotels of Arba Minch town. Among collected Datta samples, three of them have red color; the other three of them have green color while the others have gray and brown color. According to information collected from the Datta owners of each hotels, the Datta sample were prepared mainly from Chile pepper while some other spices such as garlic, ginger, coriander, and other locally available plant spices were added. The average storage time after preparation of the Datta samples were 2 weeks with a range of 3 days to the 30 days. About one third of the Datta samples collected from low profile hotel were stored at room temperature while the rests were stored in Refrigerator.

### Antimicrobial activity of Datta extract by well diffusion assay

The collected Datta samples were subjected to four solvent extractions particularly with Acetone, Ethanol, Methanol and distilled water. Stock concentration of 100mg/L Datta extract was used for antimicrobial analysis by agar well

diffusion assay against two set of bacteria. The test organisms were both multi drug resistant clinical isolates and standard (ATCC) bacteria. There is no significance difference in antimicrobial activities among the three profile hotels (P=0.93).

TABLE 1: ZONE OF INHIBITION OF DATTA SAMPLE AGAINST ATCC AND MULTI-DRUG RESISTANT CLINICAL BACTERIAL ISOLATES (100 MG/L).

Datta Source	Extract solvent	Zone of inhibition (mm) (Mean ± SD).					
		ATCC Bacteria			MDR Clinical isolates Bacteria		
		<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
HLH	Water	14.3±3.5	-	-	-	-	-
	Acetone	23.7±1.5	12.7±0.8	13*	18.3±3.6	9±5.8	19.3±1.8
	Ethanol	22±3	15.2±1	7±4.3	15.7±1.5	8.3±5.1	16.7±1
	Methanol	20.7±2.3	15.3±1.1	11.3±1	13±1.8	8±4.9	16.3±2.3
MLH	Water	-	-	-	-	12*	-
	Acetone	18.7±2.2	14.3±2	13.5**	19.3±2.7	11*	15±2.1
	Ethanol	21.3±3.9	16.3±1.6	12*	18.7±3.5	10±6.1	14±0
	Methanol	20.7±3	14.3±1	15.7±0.4	18.3±2.2	11.7±0.8	15±1.1
LLH	Water	9.3±2.4	-	-	-	-	11*
	Acetone	26.3±0.4	14.3±0.8	12±1.2	16.3±3.6	12.5**	14.3±0.8
	Ethanol	21.3±1.4	12±0.7	12.0**	16.7±0.8	8±4.9	16.7±1
	Methanol	21.3±2.4	12.7±1	12.7±1	15±1.4	11*	16.7±4.2

HLH= high level hotel MLH=medium level hotel LLH=low level hotel

\*antimicrobial activity was observed only in one sample, \*\*antimicrobial activity was observed only in two samples

The water based extract of Datta sample were exhibited significantly low antimicrobial activities (P=0.000) as compared to the other extraction solvents. The highest (14.3±3.5) zone of inhibition of water based extract was observed in high level hotel (HLH) Datta sample followed by medium profile hotel (MLH) against ATCC® 25923™ *S. aureus*. Only one sample of the medium, and low profile hotels showed antimicrobial activity against clinical isolates *E. coli* and *S. aureus* respectively (Table 1). The alcohol based (ethanol and methanol) Datta extract showed no difference in antimicrobial activities (P=0.49) in agar well diffusion assay. The highest 26.3±0.3 zone of inhibition of Acetone based extract was observed in LLH against ATCC® 25923™ *S. aureus* whereas the minimum was exhibited against *Salmonella enteritidis* (ATCC-1331). Methanol based extract showed overall average of 21, 16, and 15.4mm zone of inhibition against ATCC-25923 *S. aureus*, Methicillin-resistant *S. aureus* (MRSA), and clinical isolate *Pseudomonas speciosus* respectively. Acetone based extract exhibited 23, 18, and 16.2mm average inhibition zone against ATCC *S. aureus*, clinical isolate *Pseudomonas spp.*, and

MRSA respectively (Table 1). Pair wise comparison of ANOVA this study showed that the test organisms' susceptibility against Datta samples extract varies. As a whole ATCC *S. aureus* was more susceptible than the other test organisms (P<0.05). Similarly there is significant difference between ATCC *Salmonella enteritidis* and clinical isolate *Pseudomonas spp.* (p=0.028), and ATCC *Salmonella enteritidis* and MRSA (P=0.037). On the other hand clinical isolate *Pseudomonas spp.* showed significant difference with ATCC *S. enteritidis* and clinical isolate *E. coli* whereas *E. coli* susceptibility varies with MRSA as well (P=0.036).

### Antimicrobial activity of Chili Pepper extract by well diffusion assay

Similar to Datta samples, fresh chili pepper (*Capsicum frutescens*) family name of Solanaceae was extracted in six different extraction solvents (acetone, ethyl acetate, chloroform, ethanol, methanol and distilled water) and evaluated for antimicrobial activity by agar well diffusion assay. No zone of inhibition was observed for water based chili extract against

all test organisms at 100mg/L. All other extracts showed different zone of inhibition on test bacteria. The largest average zone of inhibition (28mm) was observed by acetone based extract against ATCC *S. aureus* while the smallest (11mm) zone of inhibition was obtained in methanol based extract against clinical isolate

MDR *P. aeruginosa*. Like in the case of Datta extract overall result showed that ATCC *S. aureus* was the most susceptible followed by clinical isolate MDR *P. aeruginosa* whereas ATCC *S. enteritidis* and MRSA were the most resistant bacteria for chili extracts (Table 2).

TABLE 2: ZONE OF INHIBITION OF CHILLI EXTRACTS AGAINST ATCC AND MULTI-DRUG RESISTANT CLINICAL BACTERIAL ISOLATES (100 MG/L)

Extract solvent	Zone of inhibition (mm)					
	ATCC Bacteria			MDR Clinical isolates Bacteria		
	<i>S. aureus</i>	<i>K. pneumonia</i>	<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
Water	-	-	-	-	-	-
Acetone	28	19	15	21	17	17
Ethanol	27	18	12	19	18	12
Methanol	24	12	13	11	16	13
Ethyl Acetate	26	12	12	15	17	15
Chloroform	23	13	17	22	13	12

Antimicrobial activities of chili extract differ based on extraction solvent. Chili Pepper water based extract showed week antimicrobial activity than the other solvents (p=0.000) similar to Datta extract. ANOVA pair wise comparison also indicated significance difference between acetone and methanol. Acetone is better extraction solvent than distilled water and methanol while there was no significant difference with other solvents. As a whole there was no significant difference (P=0.12) in susceptibility of test bacteria for chili extracts.

**Minimum inhibitory and minimum bactericidal concentration of Datta extract**

Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) for ethanol and acetone based extract were performed by four serially diluted of Datta extracts. The result of MIC of Datta extract in both extractions solvent ranges from 25 mg/L to 66.7 mg/L. Similarly, the MBC of the extracts indicates the range from 25mg/L to 100m/L. The overview of the finding showed relatively minimum concentration was required to inhibit or kill ATCC *S. aureus* than the other test bacteria.

TABLE 3: MINIMUM INHIBITORY AND MINIMUM BACTERICIDAL CONCENTRATION OF DATTA SAMPLE

Datta Source	MIC/ MBC	Extract solvent	MIC and MBC (mg/L)					
			ATCC Bacteria			MDR Clinical isolates Bacteria		
			<i>S. aureus</i>	<i>K. pneumonia</i>	<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
HLH	MIC	Ethanol	25±0	33.3±10.2	66.7±20.4	41.7±10.2	41.7±10.2	58.3±16.6
		Acetone	33.3±10.2	41.7±10.2	25±0	25±0	25±0	33.3±10.2
	MBC	Ethanol	33.3±10.2	33.3±10.2	83.3±20.4	33.3±10.2	50±0	66.7±20.4
		Acetone	58.3±27	75±10.3	25±0	41.7±10.2	41.7±10.2	50±0
MLH	MIC	Ethanol	33.3±10.2	33.3±10.2	50±0	50±0	41.7±10.2	25±0
	MBC	Acetone	41.7±10.2	33.3±10.2	41.7±10.2	33.3±10.2	25±0	50±0
		Ethanol	58.3±27	33.3±10.2	100±0	66.7±20.4	58.3±27	33.3±10.2
		Acetone	50±0	58.3±27	75±103.8	58.3±27	33.3±10.2	83.3±10.2
LLH	MIC	Ethanol	50±0	41.7±10.2	25±0	58.3±27	25±0	66.7±20.4
		Acetone	33.3±10.2	33.3±10.2	33.3±10.2	25±0	25±0	25±0
	MBC	Ethanol	83.3±20.4	58.3±27	66.7±20.4	66.7±20.4	33.3±10.2	83.3±20.4
		Acetone	50±0	58.3±27	50±0	33.3±10.2	33.3±10.2	25±0

There was no major difference between the three profile hotels. The result shows MIC of ethanol extract range from 25mg/L to 66.7mg/L for tasted organisms and acetone extract had MIC that ranges from 25mg/L to 50mg/L test bacteria specious. The overall MBC for ethanol extract has value range from 33.3 mg/L to 100mg/L whereas acetone extract had MBC that range from 25mg/L to 83.3 mg/L (Table 3).

**Minimum inhibitory and minimum bactericidal concentration of Chili pepper extract**

For Chili Pepper extracts the minimum inhibitory and minimum bactericidal concentration was determined for five solvents. In general, ATCC *S.*

*aureus* was inhibited by minimum concentration as compared to the other test organisms. Similar to MIC, the least concentration was required to kill ATCC *S. aureus* as compared to other test bacteria specious. The overview of the result showed the chloroform based extract kill test organisms at minimum concentration than the others. The least (12.5mg/L) minimum bactericidal concentration was observed in acetone based extract against ATCC *S. aureus* and in chloroform based extract against ATCC *K. pneumonia* and *S. enteritidis*, and MDR clinical isolate *P. aeruginosa* while the highest (100mg/L) was observed in all extracts for most of test bacteria specious (Table 4).

TABLE 4: MINIMUM INHIBITORY AND MINIMUM BACTERICIDAL CONCENTRATION OF CHILLI PEPPER EXTRACT

MIC/MB C	Extract solvent	MIC/MBC (mg/L)					
		ATCC Bacteria			MDR Clinical isolates Bacteria		
		<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>
MIC	Acetone	12.5	25	25	25	50	25
	Ethanol	12.5	12.5	25	50	50	25
	Methanol	25	50	25	50	50	25
	Ethyl Acetate	25	50	12.5	12.5	50	12.5
	Chloroform	12.5	12.5	12.5	12.5	100	50
MBC	Acetone	12.5	50	50	50	100	50
	Ethanol	25	25	50	100	50	25
	Methanol	50	100	50	100	100	50
	Ethyl Acetate	50	100	25	50	50	25
	Chloroform	25	12.5	12.5	12.5	100	100

**DISCUSSION**

Collected Datta samples were characterized physically and microbiologically. The samples had three different colors: red, green, and grey. The difference in color may due to the color of precursor chili pepper which associated with harvesting time. The late harvested chili pepper has red color while the early harvested has green color [4]. In microbial assessment of collected Datta sample, only gram positive rods were observed and these bacteria may be lactobacilli which involved in fermentation of Datta [3, 4]. Lactobacilli had ability to resistant to different food preservatives and diverse intrinsic antimicrobial contents of food.

Datta extracts showed antibacterial activity against all test clinical isolates and standard culture (ATCC) bacteria with zone of inhibition that ranged from 11-27mm. Also along with

determination of antimicrobial activities of Datta, antimicrobial activities of chili extract was performed in order to observe if there is significant differences. As information obtained from the Datta collected Hotels, different ingredients such as garlic and ginger were added during preparation and these have also have antimicrobial effect by themselves [13]. From this point of view more antimicrobial activities can expect from Datta as compared to sole Chili Pepper extract. However, our finding showed that there is no significant difference between Datta extract and Chili Pepper extract (P=0.069). This may be due to variation in storage time of both extracts that may be compensated by synergetic effect of ingredients in Datta because the mean storage time of Datta sample was two weeks whereas antimicrobial activities of Chili extract were performed from fresh extracted.

Antimicrobial activities of the Datta and Chili pepper water based extract showed significantly week antimicrobial activities as compared to in cell walls degradation which can extract polar and non-polar phytochemicals. Enzyme polyphenol oxidase is also active in water based extract and this inhibits phenol activities in the extract [14]. Furthermore, alcohols, acetone, ethyl acetate and chloroform are more volatile than water. This property helps them to penetrate the cellular membrane plant materials and extract the intracellular ingredients [15]. Some of phytochemicals that soluble in these solvents include tannins, polyphenols, polyacetylenes, flavone, sterols and alkaloids [16]. In other study methanol extracts of *Capsicum frutescens* were found to be more effective against *S. aureus*, and *Salmonella typhimurium*[7]. Other study also showed ethanol extract has better antimicrobial activities against *E. coli* and *Pseudomonas specious* than Chloroform and water extracts [9]. In study conducted by Tsegaye et al, Datta showed antimicrobial activities and eliminates the growth of *E. coli* in the period of fermentation [4]. The other study also showed Datta inhibits *Salmonella specious* during fermentation period [3]. In another study, on antimicrobial activity of Australian native herb extracts, the aqueous extracts displayed weak antimicrobial activities [17]. Similarly, Weerakkody et al. finding shows that water extracts of black and red pepper had weak antimicrobial activity as compared to ethanol extracts [18].

In this study multi-drug resistant (MDR) clinical bacteria isolate, and ATCC bacteria were used to assess antimicrobial effect of the extracts. Among experimental bacterial species, ATCC® 25923™ *S. aureus* was highly susceptible to extraction solvents while gram negative bacteria *E. coli* of clinical isolate was resistant. This difference may be observed due to the difference in cell wall and cell membrane composition of both groups of bacteria. Study conducted in Iran showed similar finding on *Capsicum annuum* L ethanol extract against clinical isolated microorganisms included of *K. pneumonia*, *P. aeruginosa*, *E. coli* and *S. aureus* [8]. In current study the minimum inhibitory concentration and minimum bactericidal concentration supports the finding of agar well

## REFERENCES

1. Mortensen JM, Mortensen JE. The Power of Capsaicin. *Journal of Continuing Education* 2009; 11:8-13

other solvents. This is due to alcohols, acetones, chloroform, and ethyl acetate have high efficient

diffusion assay. In conducted in Brazil, lower minimum inhibitory concentration was observed against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* compared to our study [19]. The observed difference may be due to difference in the amount of active ingredient in a plant can vary with factors like the variety of plant, weather condition of the area, the geographic location, the season and time of harvest, soil conditions, storage conditions, and the method of preparation.

Capsaicin is the main compound in the *Capsicum frutescens* which is responsible for pungency. Chili Peppers contain phenolic compounds, flavonoids and carotenoids [20] which have antimicrobial activity, antibiotic synergism and bacterial virulence removal [21]. In study that isolated phytochemical compound of this specious of Chili Pepper, chrysoeriol was the most active compound that shows high antimicrobial activities [22].

**Conclusion:** Antimicrobial activities of collected Datta samples have no significant difference among different preparation methods. Significant difference in antimicrobial activity was observed in chili pepper and Datta sample extracted with water from the other solvents. Water was week extractor of active compounds having antimicrobial activities. As a whole standard culture of *S. aureus* was more susceptible organism while standard *S. enteritidis* and clinical isolated MDR *E. coli* were less susceptible for the extracts. There was no significant difference in antimicrobial activity of chili pepper and Datta samples extracts. The traditional use of Datta for food preservation by the local people in various part of Ethiopia is supported by this study.

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2. Thomas F, Daoust SP, Raymond M. Can we understand modern humans without considering pathogens? *Evolutionary Applications* 2012; 5:368-379

3. Idris A, Mehari T, Ashenafi M. Some microbiological and biochemical studies on the fermentation of Awaze and Datta
4. Tsegaye M, Ephraim E, Ashenafi M. Behaviour of *Escherichia coli* O157:H7 during the fermentation of Datta and Awaze, traditional Ethiopian fermented condiments, and during product storage at ambient and refrigeration temperatures. *Food Microbiology* 2004; 21: 743-751.
5. Ameya G, Gure A, Dessalegn E. Antimicrobial activity of *Echinopskebericho* against human pathogenic bacteria and fungi. *Afr J Tradit Complement Altern Med* 2016; 13:199-203
6. Buli GA, Abdella G, Engda D. Antimicrobial activity of *TavernieraAbyssinica* A. Rich against human pathogenic bacteria and fungi. *African Journal of Microbiology Research* 2015; 9:2385-2390.
7. Koffi-Nevry R, Kouassi KC, Nanga ZY, Koussémon M, Loukou GY. Antibacterial Activity of Two Bell Pepper Extracts: *Capsicum annuum* L. and *Capsicum frutescens*. *International Journal of Food Properties* 2012; 15:65-68
8. Bokaeian M, Saeidi S, Bazi S, Ghamgosha M. The Effects of *Capsicum annuum* L. extract on the control of single and dual biofilms of common pathogenic strains causing urinary tract infection. *Zahedan Journal of Research in Medical Sciences* 2014; 16:65-68.
9. Hemalatha N, Dhasarathan P. Comparative study on the antimicrobial activity of *Capsicum annum* and *Capsicum frutescens*. *International Journal of Ethnomedicine and Pharmacological Research* 2013; 1:142-147
10. Kiessling CR, Cutting JH, Loftis M, Kiessling WM, Datta AR, Sofos JN. Antimicrobial resistance of food-related *Salmonella* isolates, 1999-2000. *J Food Protect* 2002; 65: 768-773.
11. Smid EJ, Gorris LGM. Natural antimicrobials for food preservation. In: Rahman MS, editor, *Handbook of Food Preservation*. New York: Marcel Dekker press, 1999. pp. 285-308.
12. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically approved standard*. 18<sup>th</sup> ed. Pennsylvania, USA: Wayne. 2012
13. Karuppiah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiberofficinale* rhizomes against multiple drug resistant clinical pathogens. *Asian Pac J Trop Biomed* 2012; 2:597-601
14. Lapornik B, Prosek M, Wondra AG. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering* 2005; 71:214-222
15. Zhu S, Ling F, Zhang Q, Liu G, Tu X, Jiang C, Wang GX. *In vivo* anthelmintic activity of five alkaloids from *Macleayamicrocarpa*(Maxim) Fedde against *Dactylogyrusintermedius*in*Carassiusauratus*. *Veterinary Parasitology*2010; 171: 305-13
16. Cowan MM. Plant products as antimicrobial agents. *Clinical microbiology reviews* 1999; 12: 564-582
17. Dupont S, Caffin N, Bhandari B, Dykes GA. *In vitro* antibacterial activity of Australian native herb extracts against food-related bacteria. *Food Control* 2006; 17: 929-932.
18. Weerakkody NS, Caffin N, Turner MS, Dykes GA. *In vitro* antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control* 2010; 21:1408-1414.
19. Nascimento PLA, Nascimento TCES, Ramos NSM, Silva GR, Câmara CA, Silva TMS, Moreira KA, Porto ALF. Antimicrobial and antioxidant activities of pimentamalagueta (*Capsicum frutescens*). *Afr J Microbiol Res* 2013; 7:3526-3533.
20. Alvarez-Parrilla E, Rosa LA, Amarowicz R, Shahidi F. Antioxidant activity of fresh and processed jalapeño and Serrano peppers. *J Agric Food Chem* 2011; 59:163-173.
21. Cushnie TPT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. *Int J Antimicrob Agents* 2011; 38:99-107.
22. Nascimento PLA, Nascimento TCES, Ramos NSM, Silva GR, Galindo GJE, Falcão REA, Moreira KA, Porto ALF, Silva TMS. Quantification, antioxidant and antimicrobial activity of phenolics isolated from different extracts of *Capsicum frutescens* (PimentaMalagueta). *Molecules*2014; 19:5434-5447.

## ORIGINAL ARTICLE

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### BACTERIAL CONTAMINATION OF WHITE COATS AND HANDS OF HEALTHCARE WORKERS AT MANSOURA UNIVERSITY CHILDREN'S HOSPITAL, MANSOURA-EGYPT

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**Running Title:** WHITE COATS AND HANDS CONTAMINATION OF HCWS AT MANSOURA UNIVERSITY

#### ABSTRACT

**Background:** Transmission of hospital acquired infections (HAIs) may be associated with contamination of healthcare workers' (HCWs) hands and white coats.

**Objective:** The purpose of this study was to clarify the role of HCWs' white coats in transmitting HAIs and to determine the association between bacterial contamination of HCWs' hands and white coats.

**Methods:** A total of 154 HCWs were enrolled in the study; different samples were taken from their hands and white coats. Samples were processed and both microbiological and biochemical characterization of the isolates were done using standard microbiological protocols.

**Results:** Up to 65.6% of hands and 61% of coats of HCWs were contaminated by microorganisms. *Staphylococcus aureus* was the most commonly isolated organisms from both hands and coats of HCWs (29.2%, 27.3% respectively) followed by MRSA (22.1%, 24.7% respectively).

**Conclusions:** The risk for contamination of hands and coats of HCWs is high in different clinical settings. In order to reduce the rate of HAIs, a strict dress protocol should be set into play to prevent cross contamination between HCWs and patients.

**Keywords:** contamination, HCWs, coat, hand, *Staphylococcus aureus*, MRSA.

### CONTAMINATION BACTÉRIENNE DE MANTEAUX BLANCS ET DE MAINS DE SOINS DE SANTÉ À L'HÔPITAL POUR ENFANTS DE L'UNIVERSITÉ MANSOURA, MANSOURA-ÉGYPTE

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**Titre en cours:** MANTEAUX BLANCS ET CONTAMINATION DES MAINS DE LA HCWS À L'UNIVERSITÉ MANSOURA

**ABSTRACT** nosocomiales (IASS) peut être associée à la contamination des mains des travailleurs de la santé et des blouses blanches.

**Contexte:** La transmission des infections

**Objectif:** Le but de cette étude était de déterminer l'étendue, le type et l'association entre la contamination bactérienne des mains des travailleurs de la santé et les blouses blanches.

**Méthodes:** Au total, 154 travailleurs de la santé ont été inclus dans l'étude; différents échantillons ont été pris de leurs mains et des manteaux blancs. Les échantillons ont été traités et la caractérisation microbiologique et biochimique des isolats a été effectuée en utilisant des protocoles microbiologiques standard.

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**Résultats:** Jusqu'à 65,6% des mains et 61% des couches de TS ont été contaminées par des micro-organismes. Le *Staphylococcus aureus* était le plus souvent isolé des deux mains et des deux sexes (29,2%, 27,3%), suivi par le SARM (22,1%, 24,7% respectivement).

**Conclusions:** Le risque de contamination des mains et des couches de TS est élevé dans différents contextes cliniques. Afin de réduire le taux d'IASS, un protocole vestimentaire strict devrait être mis en place pour prévenir la contamination croisée entre les travailleurs de la santé et les patients.

**Mots clés:** contamination, agents de santé, manteau, main, *Staphylococcus aureus*, SARM

## INTRODUCTION

Hospital acquired infections (HAIs), previously known as nosocomial infections, are those which are not present or incubated before admission of patient to the hospital but obtained during the patient's stay in hospital. The prevalence of these infections is estimated to be 5 to 10% in developed countries and 25% in developing countries.<sup>1</sup> Although the main form of transmission of HAIs is through health care workers' (HCWs) contaminated hands, HCWs' garments as coats and uniforms may play an important part in transmitting pathogenic bacteria within healthcare settings.<sup>2</sup> Within healthcare facilities, pathogenic bacteria may be transferred from patient to patient or from patient to the HCWs or the reverse.<sup>3</sup> Classically, standard infection control precautions have emphasized on hand hygiene and personal protective equipment to disrupt the chain of infection in healthcare settings.

The HCWs' coats can get contaminated by microorganisms due to improper handling practices. They get easily contaminated from infectious microorganisms that are continuously dispersed by patients in the hospital environment.<sup>4</sup> However, the role of bacterial contamination of uniforms of HCWs in the horizontal transmission of bacteria remains poorly understood.

There are accumulating data about incriminating HCWs' uniforms as a possible source of pathogenic microorganisms. These contaminated uniforms play a role in transmitting bacteria from patients to HCWs and the other way around.<sup>5</sup> Furthermore, white coats are currently implicated in transmitting the potentially pathogenic multi-drug resistant type microorganisms.<sup>6</sup>

In recent years, there was an increasing attention to prevent HAIs to reduce costs, morbidity and mortality as well as to control the problem of antibiotic resistance.<sup>7</sup> Our aim was to clarify the role of HCWs' white coats in transmitting HAIs and to determine the association between bacterial contamination of HCWs' hands and white coats.

## MATERIAL AND METHODS

We have conducted a prospective cross sectional study during the period between August 2016 and June 2017 in Mansoura University Children Hospital. The study was a conjoined effort between Microbiology and Medical Immunology Department and the Infection Control Committee at Mansoura University Children Hospital. All the participating

HCWs were informed about the study and informed consent was taken. The study protocol has been approved by Faculty of Medicine - Mansoura University ethical committee.

## Samples Collection

Samples were collected from the participating HCWs' hands and white coats across intensive care units (ICUs), internal wards and outpatients' clinics in Mansoura University Children Hospital. In addition to obtaining samples, data were collected including date, unit and profession of the HCWs. None of the approached HCWs refused to participate in this study.

We obtained swabs from the hands of the HCWs as well as from their white coats. Four predetermined sites of the white coats were sampled: the sleeve ends, the front panel at the level of the chest, the upper part of pocket and the collar. The collection of microbiological samples from coats was performed by rolling a sterile swab moistened in sterile saline on the target site.

## Culture and Identification

All swabs were cultured immediately using blood agar plates. All plates were aerobically incubated at 37 °C for 24 hours. Gram staining was used to examine the morphology and staining reaction of the organisms. Gram-negative organisms were then sub-cultured on MacConkey agar plates. Biochemical evaluation included testing for catalase, coagulase, oxidase, triple sugar iron, indole and citrate using standard protocols for identification and characterization of microorganisms. Methicillin resistant *Staphylococcus aureus* (MRSA) species were identified by using cefoxitin and oxacillin disks on Mueller Hinton agar.<sup>8</sup> Kirby Bauer's disc diffusion method was used to determine the antibiotic sensitivity of bacterial isolates.<sup>8</sup>

We classified isolated bacteria into pathogens (including *Staphylococcus aureus* (*S. aureus*), any Gram-negative bacilli and *Candida*) and skin flora (including coagulase-negative *Staphylococci*, *Bacillus* species and *Diphtheroids*).

## Statistical Analysis

Statistical analysis was performed using the SPSS statistical package software for windows version 22 (SSPS Inc, Pennsylvania, USA). Differences between two categorical variables were evaluated using Chi-

square test while the one-way analysis of variance (ANOVA) was used to determine any statistically significant differences between three variables. P value < 0.05 was considered significant difference.

## RESULTS

Of the study participants, 31 (20.1%) were doctors, 105 (68.2%) were nurses, and 18 (11.7%) were other professions (e.g., technicians, therapists). A total of 154 HCWs hands were swabbed in different ICUs, ward and outpatients' clinics (Table 1).

Bacterial growth was detected on 100 (64.9%) HCWs' hands; 45 (29.2%) grew *S. aureus*, 34 (22.1%) grew MRSA, 4 (2.6%) grew Gram-negative bacilli and 6 (3.9%) grew normal skin flora. While bacterial growth was detected on 92 (59.7%) HCWs' white coats; 42 (27.3%) grew *S. aureus*, 38 (24.7%) grew MRSA and 1 (0.6) grew Gram-negative bacilli. Hand cultures revealed Gram-positive cocci in 79 samples (51.3%) while 80 (52%) coat samples recovered Gram-positive cocci. Negative cultures were revealed from 54 (35.1%) hand swabs and 62 (40.3%) coat samples (Table 2).

**TABLE (1): DISTRIBUTION OF SAMPLES IN DIFFERENT CLINICAL SETTING AND AMONG DIFFERENT HCWS**

Place	ICU No (%)	Wards No (%)	Outpatients' clinic No (%)	Total No (%)
Doctor	11 (7.1)	9 (5.8)	11(7.1)	31 (20.1)
Nurse	46 (29.9)	46 (29.9)	13 (8.4)	105 (68.2)
Other HCWs	1 (0.6)	14 (9.1)	3 (1.9)	18 (11.7)
<b>Total</b>	<b>58 (37.7)</b>	<b>69 (44.8)</b>	<b>27 (17.5)</b>	<b>154 (100)</b>

**TABLE (2): BACTERIA ISOLATED FROM HCWS' HANDS AND WHITE COATS**

Isolated Bacteria	Hand No=154 (%)	Coat No=154 (%)
No growth	54 (35.1)	62 (40.3)
<i>S. aureus</i>	45 (29.2)	42 (27.3)
MRSA	34 (22.1)	38 (24.7)
Bacillus	5 (3.2)	6 (3.9)
Diphtheroids	1 (0.6)	0
Klebsiella	4 (2.6)	0
Pseudomonas	0	1 (0.6)
MRSA + Bacillus	3 (1.9)	2 (1.3)
<i>S. aureus</i> + Bacillus	5 (3.2)	1 (0.6)
Klebsiella + Enterococci	1 (0.6)	0
<i>S. aureus</i> + Candida	1 (0.6)	1 (0.6)
MRSA + Candida	1 (0.6)	0
MRSA + Pseudomonas	0	1 (0.6)
<b>Total</b>	<b>154 (100)</b>	<b>154 (100)</b>

**TABLE (3): CONTAMINATION RATES OF SAMPLES AMONG DIFFERENT HCWS**

Variable	Doctors (n=31)	Nurses N=(105)	Others N=(18)	P
Hands	8 (25.8)	27 (25.7)	2(11.1)	0.23
Coats	6(19.4)	21(20.0)	2(11.1)	0.12
Hands & Coats	12(38.7)	44(41.9)	7 (38.9)	0.74
Neither	5(16.1)	13 (12.4)	7 (38.9)	0.02

In our study, 63 (40.9%) HCWs had microorganisms on their hands and white coats at the same time; and only 25 (16.2%) HCWs were negative for both their hands and coats. Bacterial contamination was found on the hands of doctors and nurses more than those of other HCWs but the difference did not reach a statistical significance, (25.8, 27.7 and 11.1 percent respectively, P=0.23). Similarly, the coats of doctors and uniforms of nurses were found to be contaminated with bacteria more than coats of other HCWs and the difference did not reach a statistical significance (19.4, 20.0 and 11.1 percent respectively, P=0.12). The combination of both hands and coats of all the HCWs were almost contaminated equally, P=0.74. Nevertheless, the percentage of other HCWs who found to be negative for bacterial contamination was significantly higher than those of doctors and nurses, p=0.02 as shown in Table 3.

**TABLE (4): CONTAMINATION RATES OF SAMPLES IN DIFFERENT CLINICAL SETTING**

Variable	Inpatients (n=127)	Outpatients (n=27)	P
Hands	26 (20.5)	11 (40.7)	0.02
Coats	24 (18.9)	5(18.5)	0.89
Hands & Coats	53 (34.4)	10 (37.0)	0.68
Neither	24(18.9)	1 (0.6)	0.001

Inpatients includes ICU and ward

The hands of the HCWs in the outpatients' clinics were significantly contaminated with bacteria more than hands of HCWs in the inpatient wards and ICU, 40.7 vs 20.5, P=0.02. However, the percentage of contaminated coats and contaminated both hands and coats did not show any statistical significance P=0.89 and P=0.68 respectively. Health care workers hands and coats in the inpatient wards and ICU that did not show any bacterial contamination were significantly higher than those of outpatients, 18.9 vs 0.6, P=0.001 as shown in Table 4.

## DISCUSSION

White coat is a symbol of identification for HCWs and is considered to be a sign of protection for our patients. However, they may act as a potential source

in the transmission of pathogenic bacteria, including multidrug resistant types, in different hospital settings.<sup>9</sup>

In our study, the hands of the HCWs in the outpatients' clinics were significantly contaminated with bacteria more than hands of those in the inpatient wards and ICU, that may be explained with busy working environment in the outpatients set up and insufficient time for proper implementation of infection control measures between examined patients who continuously detach off infectious bacteria compared to inpatient wards and ICU. As the HCWs attend to patients without proper hand washing carries a great possibility of bacterial contamination and subsequently cross-contamination to other patients.

Lack of white coats and hands contamination among HCWS in the wards and ICU compared to outpatients' HCWs enforces our previous finding and suggests a solid relation between coats and hands contamination and lack of patient after care management that could carry a risk of HAIs in susceptible individuals.

We have shown that both doctors and nurses, who are more knowledgeable and medically experienced in implementing infection control measure, had more contaminated coats and hands than other HCWs such as technicians which might be due to nature of their work that is lacking direct contact with patients. The majority of the isolated organisms in our study were *S. aureus* and MRSA while normal skin flora was not detected on a large scale. The rate of contamination of HCWs' hands was up to 64.9%, while their white coats were contaminated in a rate of 59.7%. Similarly, *Surase et al.*, found that the rate of contamination was 79% for hands of the HCWs and 75% for their coats.<sup>10</sup> Other studies showed contamination of white coats ranging from 23% to 95%.<sup>11</sup> Therefore, white coats possess a risk of cross contamination in different healthcare settings.

Gram-positive cocci were the dominant isolated organisms, followed by Gram-negative organisms with the difference being statistically significant (p< 0.05). Gram-positive cocci have been recovered from (51.3%) and (52%) of hand and coat samples respectively making it the predominant bacterial group contaminating the hands and coats of the

HCWs. Other studies reported a similar predominance of Gram-positive cocci which might be potentially pathogenic particularly if the host is immune compromised.<sup>6,12,13</sup>

Among the Gram-positive cocci, *S. aureus* was recovered from 87 samples (hand: 45; 29.2%, coat: 42; 27.3%), while MRSA was detected in 72 samples (hand: 34; 22.1%, coat: 38; 24.7%). Fifty-four (35.1%) of the hand samples were negative, and 6 (3.9%) had skin flora only. High level of contamination of white coats with MRSA has been previously reported.<sup>11,14</sup> In a study conducted by *Surase et al*, MRSA accounted for 12% of the potential pathogens.<sup>10</sup>

Potentially infectious Gram-negative bacilli were also isolated in this study, but they were significantly lesser in number, as was reported by previous studies.<sup>15,16</sup>

Despite of multiple infection control measures implementation, the horizontal transmission of nosocomial pathogens, including multidrug-resistant types, persists which suggest the presence of an overlooked reservoir for these pathogens. Previously, contamination of HCWs' uniforms with potentially pathogenic bacteria and skin flora has been reported.<sup>17</sup> Similarly, those studies found pathogenic bacteria such as *S. aureus*, MRSA, Enterococci and Gram-negative bacilli.

A prohibition of wearing of white coats and any other long-sleeved garment while providing patient care

was recommended by the British Government in a uniform and dress code for physicians that was published in 2007. This prohibition is not applied in other developed countries such as the United States and developing countries such as Egypt. Despite the emerging evidence indicating bacterial contamination of uniforms and the lack of frequent washing of white coats, white coats banning during patient care remains a controversial issue.<sup>18</sup> However, we recommend, due to our findings, that a strict dress protocol should be implemented in order to prevent cross contamination between HCWs and patients and to guard against transmission of infection in different healthcare settings. Furthermore, HCWs should be encouraged to keep their white coats clean, to wash their hands frequently and to avoid touching their coats in particular after hand washing.

## CONCLUSION

The risk for contamination of hands and coats of HCWs is high in different clinical settings. A large proportion of HCWs' white coats and hands might be contaminated with potentially pathogenic *S. aureus* and MRSA. HCWs' coats may be an important vector for transmission of nosocomial pathogens. In order to reduce the rate of HAIs, a strict dress protocol should be set into play to prevent cross contamination between HCWs and patients.

## REFERENCES

1. Pittet D, Allegranzi B, Storr J et al. Infection control as a major World Health Organization priority for developing countries. *J Hosp Infect* 2008; 68: 285-292.
2. Fayyad Dharan S, Touveneau S, Sauvan V et al. Bacterial contamination of the hands of hospital staff during routine patient care. *Arch Intern Med* 1999; 159: 821-826.
3. Pandey A, Asthana AK, Tiwari R et al. Physician accessories: doctor, what you carry is every patient's worry. *Indian J Pathol Microbiol* 2010; 53(4): 711-713.
4. Trabulsi LR, Alterthum F. *Microbiology* 2007. Sao Paulo :Atheneu.
5. Munoz-Price LS, Dezfulian C, Wyckoff M et al. Effectiveness of stepwise interventions targeted to decrease central catheter-associated bloodstream infections. *Crit Care Med* 2012; 40(5):1464-1469.
6. Thaore S, Desai N, Srinidhi SR et al. Microbial contamination of lab coats while performing endodontic treatment. *IIOABJ* 2016; 7 (6): 1-6.
7. Boucher HW, Talbot GH, Bradley JS et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; 48: 1-12.
8. Performance standards for Antimicrobial Susceptibility testing; Twenty First Informational Supplement 2011. CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute.
9. Wong D, Nye K, Hollis P. Microbial flora on doctors' white coats. *Brit Med J* 1991; 303: 1602-1604.
10. Surase P, Nataraj G, Kuyare S et al. The Ever Increasing Reservoirs of Infection in the Health Care Environment – Time for a Sixth Moment of Hygiene. *Journal of the Association of Physicians of India* 2016; 64: 31-37.
11. Treacle AM, Thom KA, Furuno JP et al. Bacterial contamination of the health care workers' white coats. *Am J Infect Control* 2009; 37(2): 101-105.
12. Priya H, Shashidhar A, Bhat M et al. Microbial contamination of the white coats of dental staff in the clinical setting. *J Dent Res Dent Clin Dent Prospect* 2009; 3:136-40.
13. Loh W, Ng W, Holton J. Bacterial flora on the white coats of medical students. *J Hosp Infect* 2000; 45: 65-68.

14. Gaspard P, Eschbach E, Gunther D et al. Methicillin resistant *Staphylococcus aureus* contamination of HCWs' uniforms in long-term care facilities. *J Hosp Infect* 2009; 71: 170-175.
15. Zachary KC, Bayne PS, Morrison VJ et al. Contamination of gowns, gloves and stethoscopes with vancomycin resistant enterococci. *Infect Control Hosp Epidemiol* 2001; 22(9): 560-564.
16. Grabsch EA, Burrell LJ, O'Keeffe JM et al. Risk of environmental and healthcare worker contamination with vancomycin resistant enterococci during outpatient procedures and haemodialysis. *Infect Control Hosp Epidemiol* 2006; 27: 287-293.
17. Wiener-Well Y, Galuty M, Rudensky B et al. Nursing and physician attire as possible source of nosocomial infections. *Am J Infect Control* 2011; 39: 555-559.
18. Wilson JA, Loveday HP, Hoffman PN et al. Uniform: an evidence review of the microbiological significance of uniforms and uniform policy in the prevention and control of healthcare-associated infections. Report to the Department of Health (England). *J Hosp Infect* 2007; 66: 301-307.

## ORIGINAL ARTICLE

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### EVALUATING THE USE OF DEDICATED SWAB FOR RAPID ANTIGEN DETECTION TESTING IN GROUP A STREPTOCOCCAL PHARYNGITIS IN CHILDREN

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#### RUNNING TITLE: RAPID ANTIGEN DETECTION TESTING IN GAS PHARYNGITIS IN CHILDREN

#### ABSTRACT

**Background:** Group A streptococcus (GAS) is the most common and fearful bacterial cause in pediatric acute pharyngitis due to its serious complications. Several generations of rapid antigen detection tests (RADTs) have been developed to facilitate rapid detection of GAS pharyngitis. We assessed the value of using a dedicated swab for RADT rather than using the same swab for throat culture and RADT.

**Methods:** We conducted a prospective, single-center study that included children with suspected GAS pharyngitis. Paired throat swabs were taken simultaneously from each child. We dedicated one swab for RADT (RADT#1) and used the other swab to inoculate blood agar plate surface, and then immediately to process the RADT (RADT#2).

**Results:** The prevalence of GAS pharyngitis among the participants was 28% based on throat culture positive results. The RADT#1 and RADT#2 had sensitivity results of 92.9% and 84.3% respectively. Both RADT#1 and RADT#2 had 96.1% specificity.

**Conclusion:** We found that RADT resulted in a better sensitivity when one swab was dedicated for the test. Therefore, physicians are encouraged to use separate swabs for each diagnostic test when both RADT and throat culture are performed.

**Key words:** GAS, pharyngitis, rapid antigen detection test

### ÉVALUATION DE L'UTILISATION D'UN SWAB DEDICÉ POUR DES ESSAIS RAPIDES DE DÉTECTION D'ANTIGÈNE DANS LE GROUPE A PHARYNGITE STREPTOCOCALE EN ENFANTS

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#### TITRE FONCTIONNEL: TEST DE DETECTION ANTIGENNE RAPIDE DANS LA PHARYNGITE DE GAZ DANS LES ENFANTS

#### ABSTRAIT

**Contexte:** Le streptocoque du groupe A (GAS) est la cause bactérienne la plus fréquente et la plus effrayante de la pharyngite aiguë pédiatrique due à ses graves complications. Plusieurs générations de tests de détection d'antigène rapide (RADT) ont été développés pour faciliter la détection rapide de la pharyngite GAS. Nous avons évalué la valeur de l'utilisation d'un tampon dédié pour RADT plutôt que d'utiliser le même écouvillon pour la culture de la gorge et la RAD

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**Méthodes:** Nous avons mené une étude prospective et à centre unique comprenant des enfants atteints de pharyngite soupçonnée de GAS. Des écouvillons de gorge appariés ont été pris simultanément par chaque enfant. Nous avons dédié un écouvillon pour RADT (RADT # 1) et utilisé l'autre écouvillon pour inoculer la surface de la plaque d'agar de sang, puis immédiatement pour traiter le RADT (RADT # 2).

**Résultats:** La prévalence de la pharyngite GAS chez les participants était de 28% selon les résultats positifs de la culture de la gorge. Le RADT # 1 et le RADT # 2 ont des résultats de sensibilité de 92,9% et 84,3% respectivement. Les deux RADT # 1 et RADT # 2 avaient une spécificité de 96,1%.

**Conclusion:** Nous avons constaté que RADT a permis une meilleure sensibilité lorsqu'un tampon a été dédié au test. Par conséquent, les médecins sont encouragés à utiliser des écouvillons séparés pour chaque test de diagnostic lorsque la RADT et la culture de la gorge sont effectuées.

**Mots clés:** GAS, pharyngite, test rapide de détection d'antigène

## INTRODUCTION

Group A streptococcus [GAS] is considered the most prevalent and important bacteria that causes pediatric acute pharyngitis due to its serious complications (1, 2). It causes a considerable economic burden to the community due to the cost of medical care. Moreover, delay in school progress of children due to the disease is also a matter of concern. In the United States of America, GAS in children costs millions of US dollars per year (3)

The rapid and accurate diagnosis of GAS pharyngitis in children enables an early treatment with proper antibiotics reducing organism transmission in the community along with its complications (4). Furthermore, an accurate diagnosis of GAS pharyngitis allows proper use of antibacterial drugs with subsequent reduction in the potential risk of drug resistance (5,6). The clinical diagnosis of GAS pharyngitis is a challenging task as the symptoms are non-specific, and similar manifestations are also observed in other types of pharyngitis (7). Therefore, clinical scoring systems such as Centor and McIsaac scores were developed to identify the patients with GAS pharyngitis prior to prescribing antibiotics (8). Unfortunately, these clinical diagnostic tools have yielded inaccurate results, therefore performing laboratory tests is a necessity in these cases (9).

The bacterial culture of collected throat swabs using blood agar plates continues to be the gold standard laboratory test for GAS pharyngitis (10). Although throat swab culture allows further investigations such as subtyping and antimicrobial susceptibility testing, it has many limitations for example laboratory infrastructure, costs, and the lag period to obtain the result (24–48 hours) which can delay an effective management (10,11). These limitations could be a problem with low resources, as most of the patients cannot come back for another visit and management (12).

RADTs have been used for detection of GAS pharyngitis since the 1980s (13). Their quick turnaround time enables the diagnosis of GAS

pharyngitis within few minutes and thus helps clinicians decide appropriate management at their healthcare facility. In addition, it is easy to perform in both outpatient clinics and professional laboratory settings (14).

Rapid antigen detection tests have a specificity ranging from 90- 99% which is considered high, however, the sensitivity is variable, ranging from 75% to 95%, compared to the throat culture technique (15). Although RADTs have been used in many societies in America and Europe as clinical practice guidelines, (16,17). their widespread use is limited by their variable sensitivities. Owing to the reported variable sensitivity of RADTs, the international protocols emphasize utilizing throat culture as a supporting method in the case of negative RADTs to avoid missing any positive instance of GAS pharyngitis (10,17,18).

Regardless of the test method, careful sampling from the posterior pharynx and tonsils is essential for the accurate results as per the recommendations of Infectious Diseases Society of America (11). Most of the studies comparing the performance of RADT with throat culture used either a single swab to perform culture and RADT or separate swabs for each diagnostic test. However, none of the previous studies, up to the best of our knowledge, presented a parallel comparison of these two approaches from a single patient.

In the current study, we evaluated the performance of using a dedicated swab for RADTs rather than using the same swab for a throat culture and RADTs.

## MATERIALS AND METHODS

### Study Participants

We included eligible patients from the outpatient clinics of Mansoura University Children Hospital during the period from October 2014 till June 2015. The inclusion criteria were based on the Modified Centor score as clinical manifestations of GAS pharyngitis, including the absence of cough, temperature > 38°C, anterior cervical lymphadenitis and the presence of pharyngeal or tonsillar exudates

(19). We included the participants with Modified Centor score of  $\geq 2$  in our study. The patients who had a tonsillectomy or received antibiotics during the preceding week were excluded. An informed consent was obtained from at least one of the parents or legal guardians before enrollment in the study. Demographic and clinical data were also collected from the participants.

**Study Design**

We conducted a prospective, single-center study. Paired swabs, using the sterile swabs provided in RADT kit, were collected simultaneously from each child by rubbing the two swabs together against the back of the throat and tonsillar area (especially the areas of inflammation, ulceration or exudation), while avoiding contact with teeth, tongue, gums, and cheek surfaces. The swabs were placed in dry test tubes and immediately transported to the microbiology laboratory for further processing. We dedicated one swab for RADT (RADT#1) and used the other swab to inoculate blood agar plate surface, and then immediately to process the RADT (RADT#2).

**BinaxNOW® Strep A Card RADT**

We used a BinaxNOW® Strep A Card immunochromatographic test (Alere Scarborough, Inc. USA). The sample line in the card is a strip of antibody (anti-strep A), which is coated on a nitrocellulose membrane. The internal control line is formed by the anti-species antibody, which is coated on the same membrane forming the second stripe. The test was executed according to the manufacturer guidelines. The results were read within five minutes and interpreted by the presence or absence of pink to purple colored lines. A positive result indicated the detection of both sample and control lines, while a negative result showed only the control line. BinaxNOW® Strep A Card RADT is readily available in Egypt and it costs as low as 3 US dollars per patient that may be suitable for low and middle-income countries.

**Culture and Identification**

Incubation of blood agar plates (Oxoid, UK) was done at 37° C for 24 hrs. If there was no growth visible, re-incubation of the plates for further 24 hrs was done. We identified potential GAS by beta-hemolytic colonial morphology, Gram staining, catalase test and bacitracin test. The SLIDEX® Strepto Plus A latex

agglutination test (bioMérieux SA, France) was used for grouping to confirm GAS identification.

**Statistical Analysis**

Both sensitivity and specificity together with predictive values were calculated based on Greenhalgh’s formulas (20). The data were presented as numbers and percentages. Categorical variables were compared using the Chi-squared test and are presented as percentages (%). Statistical values were considered significant at a P-value is less than 0.05. All statistical data were analyzed by using version 15.0 of SPSS software package (Chicago, IL, USA).

**RESULTS**

We included 250 patients into our study. All the demographic data of the participating subjects are illustrated in Table-1. The prevalence of GAS among participating subjects with pharyngitis was 28% (70/250) based on throat culture which was considered as the gold standard in our study. Cervical lymphadenitis and the presence of pharyngeal or tonsillar exudates were significantly associated with GAS pharyngitis as shown in Table 2.

**TABLE 1: DEMOGRAPHIC FACTORS OF THE STUDY PARTICIPANTS**

Demographic factors	Total patients n = 250 (%)
<b>Gender</b>	
Male	130 (52)
Female	120 (48)
<b>Age</b>	
Age <5 years	109 (43.6)
Age > 5 years	141 (56.4)

TABLE 2: ASSOCIATION OF CLINICAL FINDINGS WITH CONFIRMED GAS PHARYNGITIS

Clinical finding	Children with GAS pharyngitis n = 70 (%)	Children without GAS pharyngitis n = 180 (%)	P value*
Absence of cough	55 (78.6)	150 (83.3)	0.81
Fever (temperature > 38°C)	60 (85.7)	151 (83.9)	0.87
Cervical lymphadenitis	47 (67.1)	34 (18.9)	0.02
Pharyngeal or tonsillar exudates	50 (71.4)	40 (22.2)	0.01

\*P value of the association of clinical findings with confirmed GAS pharyngitis

TABLE 3: PERFORMANCE CHARACTERISTICS OF RADT#1 AND RADT#2 COMPARED WITH THROAT CULTURE

	Culture- positive, assay- positive (n)	Culture- negative, assay- positive (n)	Culture- positive, assay-negative (n)	Culture- negative, assay- negative (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RADT#1	65	7	5	173	92.9	96.1	90.3	97.2
RADT#2	59	7	11	173	84.3	96.1	89.4	94

PPV: positive predictive value; NPV: negative predictive value

The performance of RADT#1 and RADT#2, compared with the throat culture, are shown in Table-3. Out of the 70 patients with culture confirmed GAS pharyngitis, RADT#1 and RADT#2 were truly positive in 65 and 59 patients, respectively. The RADT#1 and RADT#2 resulted in 5 and 11 false negative results, respectively. The RADT#1 sensitivity (92.9%) was considerably higher than that of RADT#2 (84.3%) however the difference was found to be statistically not significant. Both RADT#1 and RADT#2 assays had 96.1% specificity. The RADT#1 and RADT#2 had positive predictive values (PPV) of 90.3% and 89.4%, respectively, and negative predictive values (NPV) of 97.2% and 94%, respectively.

## DISCUSSION

Streptococcal pharyngitis has drawn medical attention over the years, particularly because of its potential serious problems such as post-streptococcal autoimmune sequelae. The prevalence of GAS among the study participants with pharyngitis was found to be 28%, which is almost similar to an earlier reported prevalence in Egypt (12). Earlier studies reported that the prevalence of GAS pharyngitis varies from one region to another, reaching up to 41% in some regions. This may be due to the influence of several

regional factors such as the school crowdedness level, basic sanitation, and the efficiency of healthcare systems (12,21-23). Among the clinical manifestations of the disease, tender anterior cervical lymphadenitis and the presence of pharyngeal or tonsillar exudates were significantly associated with GAS pharyngitis, which is consistent with other reports (23,24). Although the sensitivity difference between the RADT#1 and RADT#2 did not reach a statistical significance, the RADT#1 had a considerable better sensitivity than RADT#2 (92.9% versus 84.3%). The false negative results in the case of RADT#2 were more than two-fold higher than those obtained in RADT#1. This lower sensitivity of RADT#2 could be accounted for by an insufficient antigen extraction from the swab after plate inoculation, particularly if the collected swab has a low bacterial load. Such findings were also supported by low colony counts of GAS noticed with the samples that gave false negative results by RADT#2, although truly identified by RADT#1. Previous studies have also reported that the performance of RADTs is directly proportional to the bacterial load present on the collected swab (25,26). Furthermore, a low number of GAS colonies was previously noticed in the patients with false negative RADT results reflecting low bacterial load in the collected swabs (15). The sensitivity difference

between the RADT#1 and RADT#2 could also be attributed to a faulty technique and the interpretation of the RADT results. However, in this study, the processing of swabs from each patient was done by the same trained person to eliminate any user bias in the method. Both RADT#1 and RADT#2 assays had the same specificity of 96.1% that was close to previously reported data (23,27,28).

In some cases, a GAS asymptomatic carrier can be mistakenly identified for the illness that is caused by other organisms; this might be considered a study limitation. However, the discrimination between acute GAS pharyngitis and GAS carriers with acute viral pharyngitis cannot be achieved by either conventional throat culture or RADTs. Therefore, it is acceptable to treat GAS infection based on positive result of either throat culture or RADTs (15).

**CONCLUSION:** BinaxNOW Strep-A Card is a simple and quick test that can be used clinically to reduce the unnecessary use of antibiotics in children with pharyngitis. We found that RADT resulted in a better sensitivity when one swab was dedicated for the test. Therefore, physicians are encouraged to use separate swabs for each diagnostic test when both RADT and throat culture are performed.

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## REFERENCES

- 1- **Bisno AL.** Acute pharyngitis. Etiology and diagnosis. *Pediatrics* 1996. 97(6): 949-954.
- 2- **Pichichi ME.** Group A Streptococcal tonsillopharyngitis: Cost-effective diagnosis and treatment. *Ann. Emerg. Med* 1995. 25(3): 390-403.
- 3- **Pfoh E, Wessels MR, Goldmann D, Lee GM .** Burden and economic cost of group A streptococcal pharyngitis. *Pediatrics* 2008. 121(2): 229-234.
- 4- **Shulman ST.** Acute streptococcal pharyngitis in pediatric medicine: current issues in diagnosis and management. *Pediatr. Drugs* 2003. 5(1): 13-23.
- 5- **Smeesters PR, Campos D Jr, Van Melder L, De Aguiar E, Vanderpas J, Vergison A.** Pharyngitis in low-resources settings: a pragmatic clinical approach to reduce unnecessary antibiotic use. *Pediatrics* 2006. 118(6): e1607-e1611.
- 6- **Joachim L, Campos D Jr, Smeesters PR.** Pragmatic scoring system for pharyngitis in low-resource settings. *Pediatrics* 2010. 126(3): e608-e614.
- 7- **Lin MH, Fong WK, Chang PF, Yen CW, Hung KL, Lin SJ.** Predictive value of clinical features in differentiating group A beta-hemolytic streptococcal pharyngitis in children. *J. Microbiol. Immunol. Infect* 2003. 36(1): 21-25.
- 8- **Centor RM, Witherspoon JM, Dalton HP, Brody CE, Link K.** The diagnosis of strep throat in adults in the emergency room. *Med. Decis. Making* 1981. 1(3): 239-246.
- 9- **Cohen, R, Levy C, Ovetchkine P, et al.** Evaluation of streptococcal clinical scores, rapid antigen detection tests and cultures for childhood pharyngitis. *Eur. J. Pediatr* 2004. 163(4): 281-282.
- 10- **Pickering LK.** Redbook: Report of the Committee on Infectious Diseases, 27th ed., Elk Grove Village, IL: American Academy of Pediatrics 2006. 1: 612-613.
- 11- **Bisno AL, Gerber MA, Gwaltney JM Jr, Kaplan EL, Schwartz RH.** Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. *Infectious Diseases Society of America. Clin. Infect. Dis* 2002. 35(2):113-125.
- 12- **Rimoin AW, Walker CL, Hamza HS, et al.** The utility of rapid antigen detection testing for the diagnosis of streptococcal pharyngitis in low-resource settings. *Int. J. Infect. Dis* 2010. 14(12): e1048-e1053.
- 13- **Dunne EM1, Marshall JL, Baker CA, et al.** Detection of group a streptococcal pharyngitis by quantitative PCR. *BMC Infect. Dis* 2013. 13: 312-318.
- 14- **Clegg HW, Dallas SD, Roddey OF, et al.** Presbyterian Pediatric Research Group. Extrapharyngeal group A Streptococcus infection: diagnostic accuracy and utility of rapid antigen testing. *Pediatr. Infect. Dis. J* 2003. 22(8): 726-731.
- 15- **Gerber MA, Shulman ST.** Rapid diagnosis of pharyngitis caused by

- group A streptococci. Clin. Microbiol. Rev 2004. 17(3): 571-80.
- 16- **Pelucchi C, Grigoryan L, Galeone C, et al.** ESCMID Sore Throat Guideline Group . Guideline for the management of acute sore throat. Clin. Microbiol. Infect 2012. 18(1): 1-28.
- 17- **Shulman ST, Bisno AL, Clegg HW, et al.** Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin. Infect. Dis 2012. 55(10): 1279-1282.
- 18- **Dajani A, Taubert K, Ferrieri P, Peter G, Shulman S.** Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, the American Heart Association. Pediatrics 1995. 96(4): 758-764.
- 19- **McIsaac WJ, White D, Tannenbaum D, Low DE.** A clinical score to reduce unnecessary antibiotic use in patients with sore throat. Canadian Medical Association Journal 1998. 158(1): 75-83.
- 20- **Greenhalgh T.** How to read a paper: papers that report diagnostic or screening tests. British Medical Journal 1997. 315(7107): 540-543.
- 21- **Al-Najjar FY, Uduman SA.** Clinical utility of a new rapid test for the detection of group A Streptococcus and discriminate use of antibiotics for bacterial pharyngitis in an outpatient setting. Int. J. Infect. Dis 2008. 12(3): 308-311.
- 22- **Flores Mateo G, Conejero J, Grenzner Martinel E, et al.** Early diagnosis of streptococcal pharyngitis in paediatric practice: Validity of a rapid antigen detection test. Aten. Primaria 2010. 42(7): 356-361.
- 23- **Ba-Saddik IA, Munibari AA, Alhilali AM, et al.** Prevalence of Group A beta-haemolytic Streptococcus isolated from children with acute pharyngotonsillitis in Aden, Yemen. Trop. Med. Int. Health 2014. 19(4): 431-439.
- 24- **Giesecke KE, Roe MH, MacKenzie T, Todd JK.** Evaluating the American Academy of Pediatrics diagnostic standard for *Streptococcus pyogenes* pharyngitis: backup culture versus repeat rapid antigen testing. Pediatrics 2003. 111(6): e666-670.
- 25- **Kurtz B, Kurtz M, Roe M, Todd J.** Importance of inoculum size and sampling effect in rapid antigen detection for diagnosis of Streptococcus pyogenes pharyngitis. J. Clin. Microbiol 2000. 38(1): 279-281.
- 26- **Lasseter GM, McNulty CA, Richard Hobbs FD, Mant D, Little P.** PRISM Investigators. In vitro evaluation of five rapid antigen detection tests for group A beta-haemolytic streptococcal sore throat infections. Fam. Pract 2009. 26(6): 437-444.
- 27- **Cohen JF, Bertille N, Cohen R, Chalumeau M.** Rapid antigen detection test for group A streptococcus in children with pharyngitis. Cochrane Database of Systematic Reviews 2016. 7:CD010502.
- 28- **Gonsu HK, Bomki CM, Djomou F, et al.** A comparative study of the diagnostic methods for Group A streptococcal sore throat in two reference hospitals in Yaounde, Cameroon. Pan. Afr. Med. J 2015. 17(20): 139-145.

## ORIGINAL ARTICLE

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### ANTIFUNGAL SUSCEPTIBILITY AND TEST FOR CURE OF *CANDIDA SPECIES* AMONG VULVOVAGINAL CANDIDIASIS PATIENTS IN A SECONDARY CARE HOSPITAL, NIGERIA

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#### ABSTRACT

**Background:** Antimicrobial resistance among *Candida species* is an intense public health concern. The aim of the study was to determine the antifungal susceptibility pattern and test for cure of *Candida species* among women of child bearing age who visited the General Hospital Onitsha, Nigeria with symptoms suggestive of Vulvovaginal Candidiasis (VVC).

**Materials and Methods:** Eight hundred and seventy six female patients participated in the study of which high vaginal swabs were collected and evaluated mycologically by standard microbiological methods: microscopic examination and culture using sabouraud dextrose agar (SDA). Susceptibility of isolates to 4 antifungal agents was tested using agar dilution method. Clinico-mycological evaluation was also performed among the patients.

**Result:** Higher minimum inhibitory concentration (MIC) to azole antifungals was observed predominantly among non-albicans *Candida species* increasingly involved in VVC. The rate of mycological resolution was higher than symptomatic relief at 2 weeks after treatment with antifungal drug.

**Conclusion:** Efficacious treatment of VVC requires an adequate knowledge of the causative agents and more importantly the antimicrobial to which they exhibit high susceptibility.

**Keywords:** Vulvovaginal Candidiasis, Clinico- mycology, Antimicrobial resistance, *Candida species*

### SUSCEPTIBILITÉ ANTIFONGIQUE ET TEST POUR LA CURE D'ESPÈCES DE *CANDIDA* ENTRE LES PATIENTS DE CANDIDASES VULVOVAGINALES DANS UN HÔPITAL DE SOINS SECONDAIRES, NIGERIA

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#### ABSTRAIT

**Contexte:** La résistance aux antimicrobiens chez les espèces de *Candida* est un problème de santé publique intense. L'objectif de l'étude était de déterminer le schéma de susceptibilité aux antifongiques et le test de guérison des espèces de *Candida* parmi les femmes en âge de procréer qui ont visité l'hôpital général de Onitsha, au Nigeria, avec des symptômes suggérant une candidose vulvovaginale (VVC).

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**Matériaux et méthodes:** huit cent soixante-seize six patientes ont participé à l'étude des prélèvements vaginaux élevés collectés et évalués par mycologie par méthodes microbiologiques standard: examen microscopique et culture à l'aide de la gélose sabouraud dextrose (SDA). La susceptibilité des isolats à 4 agents antifongiques a été testée en utilisant une méthode de dilution en agar. Une évaluation clinico-mycologique a également été réalisée chez les patients.

**Résultat:** une concentration minimale minimale d'inhibition (MIC) en anatoxines azoliques a été observée principalement chez les espèces non-albicans *Candida* de plus en plus impliquées dans VVC. Le taux de résolution mycologique était plus élevé que le soulagement symptomatique à 2 semaines après le traitement par un médicament antifongique.

**Conclusion:** Un traitement efficace de la VVC nécessite une connaissance adéquate des agents causaux et, plus important encore, des antimicrobiens auxquels ils présentent une forte susceptibilité.

**Mots-clés:** Candidiase Vulvovaginale, Clinico-mycologie, Résistance Antimicrobienne, Espèces *Candida*

## INTRODUCTION

Antimicrobial resistance among *Candida species* involved in Vulvovaginal candidiasis (VVC) continues to thrive as a serious public health concern. Vulvovaginal candidiasis is a conventional gynecological opportunistic mycological infection caused by *Candida* species in the lower genital tract among females globally [1,2]. It has been ascertained that approximately 75 % of sexually active females have a minimum of one-time experienced symptomatic VVC [3].

The rise in incidence of fungal infection has resulted in an extensive use of antifungals [4] and the treatment is often carried out entirely on pragmatic basis, because vaginal cultures are not routinely taken, and susceptibility testing is scarcely done. Successful treatment of infections requires adequate information of the specific causative agent(s) and the drugs to which they are susceptible. Information available in the literature suggests that data on etiologic pattern in VVC and susceptibility to antifungal are lacking in Anambra State of Nigeria, particularly Onitsha, which is the commercial mainstay of the state.

Antifungal susceptibility testing provides an evaluation of antifungal efficacy, guaranteeing good treatment outcome, limiting the development of drug resistance and therapeutic ability of unendorsed composites [5-7].

## MATERIALS AND METHODS

### Study

The present study took place between 2008 and 2009 at an Obstetrics and Gynecology Department at General Hospital Onitsha, Nigeria. A total of 876 women of different age groups were included in this study.

### Ethical Consideration

Ethical approval of the study was sought from the Nnamdi Azikiwe University Teaching Hospital

Institutional Review and Ethics Committee in Nnewi, Nigeria. Written consent was sought from the study subjects before inclusion into the study.

### Collection of Clinical Samples

The sample: high vaginal swab was obtained for the study. The samples were collected by the clinician from the posterior fornix of the vagina with sterile Dacron cotton swab stick after dilation using a sterile speculum. Two swab samples were collected per subject.

### Microscopic Examination

The first vaginal swab was rolled in drop of potassium hydroxide (KOH) then covered with cover slip. The slide was mounted on a microscope and examined using 10x and 40x objective.

### Isolation and Identification

The second vaginal swab was inoculated onto Sabouraud dextrose agar (SDA) and incubated at 37°C and examined for growth daily for 4 days.

### Clinico-mycological Evaluation

The patients with *Candida* positive cultures were asked to come back to the hospital after two weeks of treatment with antifungal drugs recommended to them. At the follow-up visit, the same questionnaire was administered and another vaginal swab was collected and processed as done previously. Clinico-mycological evaluation was then carried out using the following rating scale:

- A. Symptom absent, culture negative = very good = 3 point score
- B. Symptom present, culture negative = Good = 2 point score
- C. Symptom absent, culture positive = fair = 1 point score

D. Symptom present, culture positive = poor = 0 point score

### Antifungal Susceptibility Testing

Susceptibility of the isolates to antifungal drugs was carried out by agar dilution technique [8] using the following antifungal drugs: Fluconazole powder (Medich Plc, England); Itraconazole powder (Hanmi Pharm, Korea); Miconazole powder (Janssen Pharmaceutical Beersse Belgium); Clotrimazole powder (Symmedic Laboratories) as follows:

#### i. Preparation of stock solution of antifungal drugs:

Stock solution of each of the drugs was prepared by dissolving 25.6mg of the powder in

100ml of sterile 1: 9 dilution (in distilled water) of Dimethyl sulphoxide, to give 100ml of 256 µg/ml. All the stock solutions were sterilized by tyndalization.

#### ii Agar Dilution Technique:

Double strength SDA was prepared and dispensed into McCartney bottles in 10ml amounts and sterilized by autoclaving at 121°C for 15 min, and allowed to cool to about 45°C. Two fold serial dilution of each of the drugs was subsequently made, in the molten agar, according to the protocol in table 1. The dilutions were up to 15th dilution (i.e. 0.008 - 128µg/ml). The content of each McCartney bottle was thoroughly mixed and poured into plate and was allowed to set.

Table 3: PROTOCOL FOR THE DILUTION OF ANTIFUNGAL DRUGS IN CULTURAL MEDIA

Tube No	Vol. of Medium	Vol. of Drug Soln*	Vol. of Water	Final Conc.
1.	10 ml	10 ml	0.000ml	128 µg/ml
2.	10ml	5ml	5.00ml	64 µg/ml
3.	10 ml	2.5ml	7.500ml	32 µg/ml
4.	10 ml	1.25ml	8.750ml	16 µg/ml
5.	10 ml	0.625ml	9.375 ml	8 µg/ml
6.	10 ml	0.3125ml	9.688ml	4 µg/ml
7.	10ml	10ml	0.00ml	2 µg/ml
8.	10ml	5ml	5.00ml	1 µg/ml
9.	10ml	2.5ml	7.500ml	0.5 µg/ml
10.	10ml	1.25ml	8.750ml	0.25 µg/ml
11.	10ml	0.62ml	9.375ml	0.125 µg/ml
12.	10ml	0.3125ml	9.688ml	0.0625 µg/ml
13.	10ml	10ml	0.00ml	0.032 µg/ml
14.	10ml	5ml	5.00ml	0.016 µg/ml
15.	10ml	2.5ml	7.500ml	0.008 µg/ml

\* Stock solutions used were: Stock solution A (Conc = 256 µg/ml) - for dilution 1-6; Stock solution B (Conc = 4 µg/ml) - for dilution 7-12; Stock solution C (Conc = 0.0625) - for dilution 13-15

### iii: Preparation and Standardization of Inoculums:

A total of 60 *Candida* isolates representing all the species identified were used for the test, as follows:

<i>Candida albicans</i>	n=39
<i>Candida glabrata</i>	n=8
<i>Candida tropicalis</i>	n=4
<i>Candida krusei</i>	n=5
<i>Candida dubliniensis</i>	n=4

*Candida albicans* ATCC 10231 (control organism).

Prior to testing, all isolates were sub-cultured on SDA plates and incubated overnight at 37°C to ensure purity and viability. Approximately five isolated colonies were picked and then suspended in sterile saline and homogenized; the turbidity of the suspension was adjusted to match that of a Mac Farland 0.5 turbidity standard using a spectrophotometer.

### iv. Inoculation of Plates:

The surface of the prepared SDA plates were dried at 37°C in the oven and each plate was divided into four segments. Each of the segments was inoculated with a loopful (0.01ml) of the standardized suspension of the organism. The organism was also inoculated into control plates (drug free).

### v. Determination of MIC:

After 24hrs incubation at 37°C, the amount of growth in the plates containing the different concentrations of antifungal agents was compared with the amount of growth in the drug- free growth control plates. The MIC was read as the lowest concentration of antifungal that totally inhibited the growth of the organism.

### INTERPRETATIVE CRITERIA

The interpretation of the results was based on the break points [9] established by the National Committee for Clinical Laboratory Standards (NCCLS) for different antifungal drugs against *Candida* species [Table 2].

TABLE 2: MIC BREAKPOINTS FOR SUSCEPTIBILITY OF CANDIDA TO ANTIFUNGAL DRUGS

Interpretation of MIC Values			
Drug	Susceptible	Intermediate(SDD)	Resistant
Fluconazole	≤ 8µg/ml	16-32µg/ml	>64µg/ml
Miconazole/ Cotrimazole	≤ 0.5µg/ml	1-4 µg/ml	>8µg/ml
Itraconazole	≤ 0.125µg/ml	0.25-0.5 µg/ml	> 1µg/ml

Key : S - D D = Dose dependent susceptibility

### STATISTICAL ANALYSIS

Statistical analysis: Chi square test, One way Analysis of Variance(ANOVA) and t- test conclude and validate the results at 0.05 level of significance.

### RESULT

Yeasts cells were seen as a small, oval cell, measuring 2-4 in diameter that were budding or single. Following clinico-mycological evaluation of the

patients, 73%, 67%, 50% and 50% of patients on Fluconazole, Miconazole, Clotrimazole and Itraconazole respectively were symptomatically relieved while 91%, 89%, 88% and 17% of patients on Fluconazole, Miconazole, Clotrimazole and Itraconazole respectively were mycologically relieved at 2 weeks after treatment [Table 3]. The rate of mycological resolution was higher than symptomatic relief at 2 weeks after treatment with antifungal drug [Table 4].

TABLE 3: CLINICO -MYCOLOGICALLY EVALUATION AT 2 WEEKS AFTER TREATMENT WITH ANTIFUNGAL DRUGS.

Drug	Number	A	B	C	D	Score
		Very Good	Good	Fair	Poor	
Admitted						
Fluconazole	11	7	3	1	0	28
Miconazole	9	5	3	1	0	22
Clotrimazole	8	3	4	1	1	18
Itraconazole	14	6	4	1	3	17

The following rating scales were used: A. Symptom absent, culture negative = Very good =3 point score; B. Symptom present, culture negative = Good = 2 point score; C. Symptom absent, culture positive = Fair = 1 point score; D. Symptom present, culture positive = Poor= Zero point score

TABLE 4: SYMPTOMATIC RELIEF AND MYCOLOGICAL CURE RATES AT 2WEEKAFTER TREATMENT WITH ANTIFUNGAL DRUGS

Drug	Symptomatic relief	Mycological cure
Fluconazole	8 (73%)	10 (91%)
Miconazole	6 (67%)	8 (89%)
Clotrimazole	4 (50%)	7 (88%)
Itraconazole	7 (50%)	10 (71%)

Mycological vs symptomatic relief at 2 weeks (t = 6.91 ;< 0.05)

The minimum inhibitory concentration (MIC) of Antifungal agents against *Candida species* isolated from women with vulvovaginal candidiasis was done using the interpretative criteria on Table 1. The susceptibility testing of 60 *Candida* isolates (representing different species) to fluconazole (*C. albicans*, *C. tropicalis*, *C. dubliniensis*, *C. krusei* and *C. glabrata*) showed varied degree of susceptibility. Of the 60 isolates, 52 (86.7%) were susceptible (MIC< 8 mg/ml) to fluconazole whereas, 8 (13.3%) isolates were intermediate with MIC 16-32 mg/ml. There was no record of resistance with fluconazole in this study [Table 5]. Of the 8 isolates with elevated MIC, 4

isolates were *Candida glabrata*, 1 isolate was *Candida tropicalis*, 2 isolates were *Candida krusei* and 1 isolate *Candida albicans*. All the 60 *Candida* isolates (100%) had very low MIC to Miconazole and Cotrimazole (< 0.5 µg/m1) [Table 6]. On the contrary, only 26 (43%) of the isolates were susceptible to Itraconazole (MIC <0.125 µg/m1), whereas 21 (35%) were resistant (MIC>1µg/m1), while 13 (22%) showed dose dependent susceptibility to the drug. Among all the species, the most resistant was the *C. tropicalis* which recorded 100% resistance to Itraconazole, followed by *C. krusei* which has about 40% resistance. [Table 7].

TABLE 5: MINIMUM INHIBITORY CONCENTRATION OF FLUCONAZOLE ON CANDIDA SPECIES ISOLATED FROM VULVOVAGINAL CANDIDIASIS PATIENTS IN ANAMBRA STATE

Candida species	MIC ( $\mu\text{g/ml}$ )		
	$\leq 8 \mu\text{g/ml}$ (Susceptible)	16-32 $\mu\text{g/ml}$ (Intermediate)	$\geq 64\mu\text{g/ml}$ (Resistance)
<i>Candida albicans</i>	38/39	1/39	0/39
<i>Candida tropicalis</i>	3/4	1/4	0/4
<i>Candida dubliniensis</i>	4/4	0/4	0/4
<i>Candida krusei</i>	3/5	2/5	0/4
<i>Candida glabrata</i>	4/8	4/8	0/4
Total (%)	52/60 (86.7%)	8/60 (13.3%)	0/60 (0%)

TABLE 6: MIC OF MICRONAZOLE/ CLOTRIMAZOLE AMONG CANDIDA SPECIES

Candida species	MIC ( $\mu\text{g/ml}$ )	
	$\leq 0.5 \mu\text{g/ml}$ (Susceptible)	$\geq 8\mu\text{g/ml}$ (Resistance)
<i>Candida albicans</i>	39/39	0/39
<i>Candida tropicalis</i>	4/4	0/4
<i>Candida dubliniensis</i>	4/4	0/4
<i>Candida krusei</i>	5/5	0/5
<i>Candida glabrata</i>	8/8	0/8
Total (%)	60/60 (100%)	0/60 (0%)

TABLE 7: MINIMUM INHIBITORY CONCENTRATION OF ITRACONAZOLE ON CANDIDA SPECIES ISOLATED FROM VULVOVAGINAL CANDIDIASIS PATIENTS IN ONITSHA, ANAMBRA STATE

Candida species	MIC ( $\mu\text{g/ml}$ )		
	$\leq 0.125 \mu\text{g/ml}$ (Susceptible)	0.25-0.5 $\mu\text{g/ml}$ (Intermediate)	$\geq 1\mu\text{g/ml}$ (Resistance)
<i>Candida albicans</i>	19/39	8/39	12/39
<i>Candida tropicalis</i>	0/4	0/4	4/4
<i>Candida dubliniensis</i>	0/4	1/4	1/4
<i>Candida krusei</i>	2/5	1/5	2/5
<i>Candida glabrata</i>	2/8	3/8	2/8
Total (%)	26/60 (43%)	13/60 (22%)	21/60(35%)

## DISCUSSION

The study compares the antifungal susceptibility patterns of 4 antifungal drugs against isolated *Candida* species. The antimicrobial susceptibility testing in this study revealed that most non albicans were resistant to Itraconazole. None of the candida isolates tested were resistant to Miconazole, Clotrimazole and Fluconazole, although susceptibility of 14% of the isolates to fluconazole was dose dependent (S-DD), and majority 7/8 (88%) of these (S-DD) were non-albicans Candida (*Candida glabrata* and *Candida krusei*). The extensive use of Fluconazole leads to a shift in the causative agents of Candida infections to non-albicans species such as *C.glabrata*, *C.krusei* and *C.tropicalis* [10]. Although the efficacy of the drugs could not be established on the basis of the invitro susceptibility report since there was a degree of variability in the MIC values within the class of antifungals. Fluconazole, Miconazole and Clotrimazole appears to be the best choice, while Itraconazole due to its lower efficacy against non-albicans candida would be a poor choice in the blind treatment of vulvovaginal candidiasis in this locality as non-albicans are increasingly important participant in vulvovaginal candidiasis in this locality.

A recent study by Kiguli *et al.*, [11] described *C. krusei* with a high resistance of 71.43% to fluconazole whereas *C. glabrata*, and *C. krusei* exhibited 100% resistance and *C. albicans* exhibiting 20.59% to itraconazole. Resistance to clotrimazole was observed in 36.67% and 0.61% of *C. glabrata* and *C. albicans* respectively. Resistance to clotrimazole and fluconazole does not correlate with our study that revealed 0% resistance respectively by all *Candida* species. While, resistance to itraconazole by *C.glabrata*, *C.krusei* and *C.albicans* partially agrees with reports from our study which revealed 20%, 40% and 30.8%

resistance to itraconazole by *C.glabrata*, *C.krusei* and *C.albicans* respectively.

Dharmik *et al.*, [12] revealed that fluconazole was highly effective against *Candida* Species (97.2 %) while, the highest resistance was observed in the case of miconazole (63 %). This is contrary to our study that observed highest resistance to itraconazole (35%).

Resistance to itraconazole was observed in 16.2% (MIC  $\geq$  1  $\mu$ g/ml). Among different species, elevated fluconazole MICs ( $\geq$ 16  $\mu$ g/ml) were only observed in *C. glabrata* (15.2% resistant [R], 51.8% susceptible-dose dependent [S-DD]), and *C. krusei* (50% S-DD, 41.7% R, considered intrinsically fluconazole resistant). Resistance to itraconazole was observed among *C. glabrata* (74.1%) and *C. krusei* (58.3%). These results by Richter *et al.*, [13] support that of this study which showed resistance to itraconazole ((MIC  $\geq$  1  $\mu$ g/ml) among *C. glabrata* (20%) and *C. krusei* (40%). Furthermore, supports elevated fluconazole MICs (16-32  $\mu$ g/ml) observed more frequently among *C.glabrata*, *C.krusei*, *C.tropicalis* and *C.albicans*. Thus, fluconazole resistance among vaginal *Candida albicans* isolates is emerging.

Comparison of symptomatic relief and mycological cure rate at second week after treatment with Fluconazole, Miconazole, Clotrimazole and Itraconazole shows that the rate of mycological cure was significantly more than the symptomatic relief (t=6.91; p < 0.05). The time required by the body to recover after the annihilation of the infecting organism could be a contributing factor. Hence, the use of azoles for empirical therapy of uncomplicated Vulvovaginal candidiasis is recommended.

## REFERENCES

19. Anderson, M., Korasz, A., Friedland, S. Are vaginal symptoms ever normal? A review of literature. *Med Gen Med.* 2004; 6:49-55.
20. Sobel, J. Vulvovaginal Candidiasis. *Lancet.* 2007; 369(9577):1961-1971.
21. Lisiak, M., Klyszejko, C., Pierzchalo, T., Marcinkowski, Z. Vaginal candidiasis: frequency of occurrence and risk factors. *Ginekol Pol.* 2003; 71:964-970.
22. Gupta, A.K., Tomas, E. New antifungal agents. *Dermatology Clinic* 2003; 21: 565 - 576.
23. Espinel-Ingroff, A. Clinical relevance of antifungal resistance. *Infect Dis Clin North Am.* 1997; 11:929-944.
24. Pfaller, M.A., Yu, W.L. Antifungal susceptibility testing. New technology and clinical applications. *Infect Dis Clin North Am.* 2001; 15:1227-1261.
25. Pfaller, M.A., Rex, J.H., Rinaldi, M.G. Antifungal susceptibility testing: Technical advances and potential clinical applications. *Clin Infect Dis.* 1997; 24:776-784.
26. Shadomy, S., Pfaller, M.A. Laboratory studies with antifungal agents: susceptibility tests and quantitation in body fluids. In :Balows, A., Hausler, W. Jr, Hermann, K., Isenberg, H.D., Shadomy, H.J. (eds) 5<sup>th</sup> ed. *Manual of Clinical Microbiology.* ASM Press, Washington DC 1991: 1173 -1181.
27. Gharnnoum, M.A., Rex, J.H., Galgiani, J.N. Susceptibility testing of fungi: Current status

- of correlation of invitro data with clinical outcome. *Journal of Clinical Microbiology*. 1996; 34: 489 - 495
28. Efushine, A.M., Oduyebo, O., Osuagwu, C.S., Koenig, B. Species distribution and antifungal susceptibility of *Candida* Isolates from pregnant women in a tertiary hospital in Nigeria. *Afr J Clin Exper Microbiol*. 2016; 17:183-189.
  29. Kiguli, J.M. , Kibuka L.S. , Bazira, J. , Byarugaba, F. Antifungal Susceptibility Patterns of Vulvovaginal *Candida* species among Women Attending Antenatal Clinic at Mbarara Regional Referral Hospital, South Western Uganda. *Br Microbiol Res J*. 2015; 5(4): 322-331.
  30. Dharmik, P.G., Gomashe, A. V., Upadhyay, V. G. Susceptibility Pattern of Various Azoles Against *Candida* Species Causing Vulvovaginal Candidiasis. *J Obstet Gynaecol*. 2013; 63(2): 135-137.
  31. Richter, S.S., Galask, R.P., Messer, S.A., Hollis, R.L., Diekema, D.L., Pfaller, M. A. Antifungal Susceptibilities of *Candida* Species Causing Vulvovaginitis and Epidemiology of Recurrent Cases. *J. Clin. Microbiol*. 2005; 43(5): 2155-2162.

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### VULVO-VAGINAL CANDIDOSIS IN A COHORT OF HORMONAL CONTRACEPTIVE USERS IN IBADAN, NIGERIA

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#### ABSTRACT

**Introduction:** Most women who acquire HIV and other sexually transmitted infections (STIs) are in their child bearing years and are current or potential users of contraceptive methods. The study was undertaken to provide information on the association between the hormonal contraceptive methods and vulvo vaginal candidiasis among women attending Family Planning clinics, University College Hospital, Ibadan, Nigeria.

**Methods:** It was a cross-sectional study in a population of women using hormonal contraceptive methods attending Family Planning clinics. Detailed medical history, endocervical and high vaginal swabs were collected from the women to establish diagnosis after clinical examination and informed consent. Aliquots of sera from venous blood samples of the women were tested for antibodies to HIV-1/2. Data was analysed using SPSS for windows' version 17.0.

**Results:** There were 116 women using hormonal contraceptive methods who participated in the study with mean age of 28.70 years (SD = 6.72, range = 19 -54). The mean age of sexual debut of the women was 19.2 years (SD = 2.96). The prevalence of vulvo-vaginal candidosis was 23.3%. Other associated sexually transmitted infections were bacterial vaginosis (24.1%), HIV (12.1%), trichomoniasis (10.3%), chlamydia cervicitis (7.8%), syphilis (5.2%), genital warts (6.0%) and gonorrhoea (2.6%). Younger age of sexual debut influenced the decision of selecting various forms of hormonal contraceptives especially the emergence of oral contraceptive forms (P = 0.043). Majority of the women on hormonal contraceptives had multiple sexual partners. Vulvovaginal candidosis is strongly associated with vaginal discharge and pruritus in women utilizing hormonal contraceptive methods (P = 0.001, 4.2 (1.0-13.2).

**Conclusions:** Women seeking contraception to prevent unintended pregnancy are as much in need of education about prevention of STIs. The study found that younger age, numbers sexual partners, and use of hormonal contraceptives could increase the risk of acquiring vulvovaginal candidosis.

### CANDIDOSE VULVO-VAGINALE DANS UNE COHORTE D'UTILISATEURS CONTRACEPTIFS HORMONAUX A IBADAN, NIGERIA

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#### ABSTRAIT

**Introduction:** La plupart des femmes qui contractent le VIH et d'autres infections sexuellement transmissibles (IST) sont en âge de procréer et sont des utilisatrices actuelles ou potentielles de méthodes contraceptives. L'étude a été entreprise pour fournir des informations sur l'association entre les méthodes contraceptives hormonales et la candidose vulvo vagin chez les femmes fréquentant les cliniques de planification familiale, University College Hospital, Ibadan, Nigeria.

**Méthodes:** Il s'agissait d'une étude transversale menée auprès d'une population de femmes utilisant des méthodes contraceptives hormonales dans des cliniques de planification familiale.

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Des antécédents médicaux détaillés, des prélèvements endocervicaux et des prélèvements vaginaux élevés ont été effectués auprès des femmes pour établir un diagnostic après un examen clinique et un consentement éclairé. Des aliquotes de sérums d'échantillons de sang veineux des femmes ont été testées pour rechercher des anticorps anti-VIH-1/2. Les données ont été analysées à l'aide de SPSS pour la version 17.0 des veuves.

**Résultats:** 116 femmes utilisant des méthodes contraceptives hormonales ont participé à l'étude avec un âge moyen de 28,70 ans (ET = 6,72, intervalle = 19-54). L'âge moyen des débuts sexuels des femmes était de 19,2 ans (ET = 2,96). La prévalence de la candidose vulvo-vaginale était de 23,3%. Les autres infections sexuellement transmissibles étaient la vaginose bactérienne (24,1%), le VIH (12,1%), la trichomonase (10,3%), la chlamydia cervicite (7,8%), la syphilis (5,2%), les verrues génitales et la gonorrhée. . L'âge plus jeune des débuts sexuels a influencé la décision de choisir différentes formes de contraceptifs hormonaux, en particulier l'émergence de formes contraceptives orales ( $p = 0,043$ ). La majorité des femmes sur les contraceptifs hormonaux avaient plusieurs partenaires sexuels. La candidose vulvovaginale est fortement associée aux pertes vaginales et au prurit chez les femmes utilisant des méthodes contraceptives hormonales ( $p = 0,001, 4,2 (1,0-13,2)$ ).

**Conclusions:** Les femmes qui recherchent une contraception pour prévenir les grossesses non désirées ont autant besoin d'éducation sur la prévention des IST. L'étude a révélé que le plus jeune âge, le nombre de partenaires sexuels et l'utilisation de contraceptifs hormonaux pourraient augmenter le risque d'acquisition de la candidose vulvo-vaginale.

## INTRODUCTION

Contraception has been recognized as an important part of national efforts in many countries of the world to reduce adolescent pregnancies (1). Avoidance of unintended pregnancies requires access to, and appropriate use of effective and safe methods of fertility control (2). This invariably leads to the improvement of reproductive health of sexually active young women (2). Unhindered access to effective and safe contraceptive methods could be the key to individual and public health safety.(3) A number of safe and effective contraceptive methods are available, and these include abstinence, barrier methods, oral contraceptives, Depo-Provera, Norplant implant, Intra-uterine devices, and sterilization methods (1).

The current or potential users of different methods of contraceptives who acquire sexually transmitted infections and Human Immunodeficiency virus infections are in their child bearing years. There is a controversy over the extent to which specific contraceptive methods increase or perhaps reduce the risk of HIV infection(4). Hormonal contraception has been proven as one of the biological factors being linked to the acquisition of Human Immunodeficiency Virus (HIV) and other sexually transmitted infections (STIs).(5, 6) In a study conducted among a cohort of female sex workers in Kenya, compared with those who don't practice contraception; those who rely on oral contraceptives (OCs) are more likely to acquire chlamydia infection or vaginal candidiasis, but are less likely to acquire bacterial vaginosis. (6, 7) The assumptions are that the cervical ectopy produced by oral contraceptives (OCs) results in affected cervical -zone being more vulnerable to trauma and thus perhaps to HIV infection.(8). Injectable hormonal contraceptives may increase the risk by increasing bleedings and thinning of the vaginal epithelium.(4) Most hormonal contraceptives contain either or both progesterone and oestrogen.(9)

Hormonal contraceptives usually include combination of estrogen and progestin; and progestin only. Estrogen effects include inhibition of ovulation and prevention of follicular maturation through suppression of ovarian steroid production and possibly decreased responsiveness to gonadotropin-releasing hormone (9). Conversely, progestin leads to changes in the endometrium that make implantation less likely, increased thickness of cervical mucus that makes sperm penetration difficult, and impairment of normal tubal mobility.(10)

In spite of recent therapeutic advances, vulvovaginal candidiasis remains a common global problem of public health importance.(11) The prevalence of vulvo-vaginal candidiasis varies worldwide, and in community-based survey conducted among female commercial sex workers in Ibadan, Nigeria, VVC was the commonest sexually transmitted infections reported (12) in women. It has been estimated that more than 70% of women develop at least one episode in their life time, (11) 50% experience a second episode, (11, 13, 14) while 5-8% encounter recurrences.(15) Oestrogen dominance usually enhances overgrowth of *Candida spp* in the vaginal milieu; and it has also been established in previous studies that oral contraceptives may determine the possibility of recurrent vulvo-vaginal candidiasis (14, 16, 17). Other predisposing factors linked to vaginal candidiasis include HIV infection, pregnancy, diabetes and undue prolonged use of antibiotics. (14, 18) Sexual behaviour could play an important role in the acquisition of VVC since some of the risk factors have been linked to the level of sexual activities with these women. Sexual behaviour has been linked to influence both primary VVC infections and relapses.(19)

Presently there is a dearth of information of the prevalence of vulvo-vaginal candidiasis and its associated risk behaviours among hormonal contraceptive users in south western Nigeria. This study was aimed at evaluating the pattern of vulvo-vaginal candidiasis among the cohorts of hormonal contraceptive

users attending the family planning clinic, University College Hospital, Ibadan.

## MATERIALS AND METHODS

This was a cross-sectional study carried out in a population of child-bearing age women using different types of hormonal contraceptive methods and attending family planning clinic, University College Hospital between March – December 2006.

### Sampling Procedure

Women were recruited into the study as they presented to the clinic consecutively and freely gave informed consents. The women that were enrolled into the study completed structured questionnaires to obtain baseline information on their socio-demographic characteristics, reproductive health history including sexual behaviours; and different types of hormonal contraceptives being used. Pre- test counselling was done for all clients recruited into the study which emphasized on client confidentiality; reasons for screening for candidiasis and HIV testing; information about their current and previous risk behaviours and also implication of positive test results. Women were excluded from being enrolled into the study if they were menstruating or have used antifungals especially the azoles in the preceding two weeks or within six weeks post-abortion.

### Physical Examination and Specimen Collection

All women who freely gave informed consent and met the inclusion criteria had a complete pelvic examination under aseptic conditions for signs and symptoms suggestive of vulvovaginal candidiasis and other signs of sexually transmitted infections (STIs). High vaginal swabs and Endocervical swabs were collected from all the participants by the attending physicians following standard procedures. These samples were transported in Amies transport medium to the Special Treatment Clinic Laboratory, University College Hospital, Ibadan for microscopy, culture and sensitivity. The colour, character and the smell of the vaginal discharge were also noted.

### Laboratory Procedures

Vaginal secretions collected were subjected to wet preparation and potassium hydroxide microscopy for the identification of round to oval budding yeast cells, trichomonads and clue cells. High vaginal secretions were also cultured on Sabouraud's dextrose agar (SDA) at 37°C for 24 to 48hrs. Saline wet preparation of the creamy-greyish colonies revealed the presence of multiple budding yeast cells and pseudohyphae suggestive of *Candida* species. Species identification was confirmed based on the results obtained from germ tube

tests, sugar assimilation and fermentation tests based on standard methods. Germ tubes positive isolates were confirmed as *Candida albicans*.

Endocervical secretions were cultured for *N. gonorrhoeae* on modified Thayer Martins Agar. The media plates were incubated at 37°C in 5% CO<sub>2</sub> humidified extinction jar. Endocervical secretions were also Gram-stained for the presence of intracellular diplococci which were later confirmed as *Neisseria gonorrhoeae* by standard laboratory methods. Endocervical secretions were also tested for *Chlamydia trachomatis* by QuickView Chlamydia test kits following standard procedures (20). Diagnosis of genital warts was based on the clinical findings of typical lesions as previously described various researchers on the external genitalia, vaginal, cervix and perianal region. (21-23)

HIV-serology testing was done by rapid immunochromatographic test strips using OraQuick ADVANCE™ rapid HIV- 1 / 2 antibody testing. Any sample positive on screening was re-tested with enzyme-linked immunosorbent assay (ELISA) and confirmed by western blot analysis. Approval for the study was obtained from UI/UCH ethics review committee.

### Data Analysis

Data analysis was done using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA). Summary statistics such as proportions for categorical variables; means and standard deviation for continuous variables were estimated. The variables included for the analysis were women's age, educational level, religion, ethnicity, marital status, occupation, age of sexual debut, numbers of sexual partners by the women, numbers of multiple partners of their spouses, types of contraceptives and prevalence of candidiasis. Frequency distribution tables for each of the variables were analysed. Odds ratio and confidence interval were calculated to evaluate the association between sexual behaviour, hormonal contraception and prevalence of candidiasis. Multivariate analysis was used to assess the effect of the confounding variables. Statistical significance was set at  $P < 0.05$ .

## RESULTS

During the study period, a total of one hundred and sixteen women attending family planning clinic at the University College hospital; that fulfilled the inclusion criteria and using different hormonal contraceptives were included in the analysis. Table 1 shows the sociodemographic characteristics and the rate of candidiasis of the women. The mean age of the women was 28.70 years (SD = 6.72, range = 19 -54). Sixty-six (66, 56.9%) were in the 20-29 age range and more than half (16 of 27, 59.3%) of this age group were infected with candidiasis. Nearly half of them also (57, 49.1%) were

secondary school leavers and fifty of them (50, 43.1%) were neither married nor living with a partner. Table 1.

with their casual friends and school mates while only 30 (25.9%) initiated sex with their spouses. (Table 2)

The mean age of sexual debut of the women was 19.2 years (SD = 2.96) Fifty-five of them had their sexual debut

**TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE PARTICIPANTS**

Characteristics	Category	Frequency (n=116) (%)	Candidiasis (n=27) (%)	P -value
<b>Age in years</b>	10-19	4 (3.4)	0 (0.0)	0.557
	20-29	66 (56.9)	16 (59.3)	
	30-39	38 (32.8)	9 (33.3)	
	40-49	6 (5.2)	2 (7.4)	
	50-59	2 (1.7)	0 (0.0)	
<b>Level of Education</b>	No formal education	2 (1.7)	0 (0.0)	0.576
	Primary school	15 (12.9)	2 (7.4)	
	Secondary school	57 (49.1)	14 (51.9)	
	Polytechnic / Colleges of Education	21 (18.1)	4 (14.8)	
	University	21 (18.1)	7 (25.9)	
<b>Marital Status</b>	Not married, Not living with a partner	50 (43.1)	10 (37.0)	0.614
	Not married, Living with a partner	9 (7.8)	1 (3.7)	
	Married , Not living with a partner	17 (14.7)	5 (18.5)	
	Married , Living with a partner	40(34.5)	11 (40.7)	
<b>Social Class</b>	Class I	2 (1.7)	1 (3.7)	0.160
	Class II	27 (23.3)	9 (33.3)	
	Class III	9 (7.8)	2 (7.4)	
	Class IV	1 (0.9)	1(3.7)	
	Class V	33 (28.4)	8 (29.6)	
	Class VI	44 (37.9)	6 (22.2)	

Choice of hormonal contraceptives was statistically significant for younger women (Mean= 22.5 years). Younger age of sexual debut also influenced the choice of hormonal contraceptives especially the emergency contraceptives pills. Table 3 shows that thirty-six (31.0%) of the women preferred injectable hormonal contraceptives while thirty (25.9%) were taking oral contraceptives. Those women on oral contraceptive had the highest rate of VVC (11 of 27,

40.1%). The odds of women on oral contraceptive having VVC are slightly higher but are not statistically significant. (Table 3)

The prevalence of vulvo-vaginal candidosis in this study was 23.3% (27 of 116), Nineteen (70.4%) were *C. albicans* while eight were non-albicans *Candida*.

TABLE 2: SEXUAL AND REPRODUCTIVE HISTORY OF THE WOMEN

Variables	Categories	Frequency N=116 (%)	Candidiasis n=27(%)	P- value
Age of sexual debut	11-15 16-20 21-25 26-30	4 (3.4) 81 (69.8) 26 (22.4) 5 (4.4)	1 (3.7) 19 (70.4) 6 (22.2) 0 (0.0)	0.457
Age of co-habiting with a partner	16-20 21-25 26-30 31-35 No cohabiting	22 (19.0) 49 (42.2) 14 (12.0) 1 (0.9) 30 (25.9)	4 (14.8) 15 (55.6) 3 (11.1) 0 (0.0) 5 (18.5)	0.934
No of sexual partners in the last one month	0 1 2 3 4	13 (11.2) 62 (53.4) 33 (28.4) 6 (5.2) 2 (1.7)	2 (7.4) 12 (44.4) 9 (33.3) 3 (11.1) 1 (3.7)	0.345
Spouses with multiple partners	Yes No Don't know	23 (19.8) 33 (28.4) 60 (51.8)	6 (22.2) 10 (37.0) 11 (40.7)	0.400

TABLE 4:

TABLE 4: PATTERN OF VULVO-VAGINAL CANDIDIASIS AND OTHER STIS AMONG WOMEN USING HORMONAL CONTRACEPTIVES.

TABLE 3: TYPES OF HORMONAL CONTRACEPTIVES

Types of Hormonal Contraceptives	Frequency (N=116)	Percentage (%)
Oral Contraceptives	30	25.9
Injectable	36	31.0
Emergency contraceptives	48	41.4
Norplant implant	2	1.7

Infections	Frequency (N=116)	Percentage (%)
Vulvo-vaginal candidiasis	27	23.3
Bacterial vaginosis	28	24.1
Trichomoniasis	12	10.3
Chlamydia trachomatis	9	7.8
Gonorrhoea	3	2.6
Genital warts	7	6.0
HIV infection	14	12.1

Other sexually transmitted infections diagnosed include bacterial vaginosis (24.1%), trichomoniasis (10.3%), *Chlamydia trachomatis* infection (7.8%), Gonorrhoea (2.6%), genital warts (6.0%) and HIV infection (12.1%) as shown in Table 4. There was no significant association between the women infected with candidiasis and acquisition of HIV infection. Five (18.5%) of the women with vulvovaginal candidiasis were diagnosed with HIV infection. (P =

0.24). However, two (66.7%) of the patients with gonorrhoea were infected with HIV infection. Gonorrhoea was found to be significantly associated with HIV acquisition  $P = 0.003$ . The odds ratio of

hormonal contraceptive users infected with gonorrhoea acquiring HIV infection was 16.8 (1.4-199.8). Other STIs were not associated with acquisition of HIV. (Table 5)

**TABLE 5: ASSOCIATION OF VULVOVAGINAL CANDIDIASIS AND OTHER STIS WITH HIV INFECTION**

Infection	HIV Infection		P Value	OR (95% CI)
	Negative Frequency (%)	Positive Frequency (%)		
Vulvo-vaginal candidiasis	22 (81.5)	5 (18.5)	0.24	2.02 (0.61-6.6)
Bacterial vaginosis	25 (89.3)	3 (10.7)	0.801	0.84(0.22-3.2)
Trichomoniasis	9 (75.0)	3 (25.0)	0.146	2.82 (0.66-11.9)
Chlamydia trachomatis	7(77.8)	2 (22.2)	0.330	2.2 (0.421-12.1)
Gonorrhoea	1(33.3)	2 (66.7)	0.003*	16.8 (1.4-199.8)
Genital warts	6 (85.7)	1 (14.3)	0.850	1.23 ( 0.137-11.05)

**TABLE 6: ASSOCIATION OF VULVO-VAGINAL CANDIDIASIS WITH HORMONAL CONTRACEPTIVES TYPE AND OTHER RISK FACTORS**

Infection	Vulvo-vaginal candidiasis		P Value	OR (95% CI)
	Positive Frequency (%)	Negative Frequency (%)		
Types of Contraceptives / Risk factors				
Oral Contraceptives	8 (26.7)	22 (72.3)	0.610	0.78 (0.30-2.03)
Injectable Contraceptives	8 (22.2)	28 (77.8)	0.857	1.06(0.426-2.78)
Norplant implant	0 (0.0)	2 (100.0)	0.432	2.82 (0.66-11.9)
Emergency Contraceptives	11 (22.9)	37 (77.1)	0.939	1.04 (0.43-2.46)
Multiple sexual partners	26 (42.6)	35 (57.4)	0.261	1.04 (0.96-1.12)
Douching	7 (16.3)	36 (83.7)	0.171	1.94 (0.74-5.06)
Vaginal discharge	23 (45.1)	28 (54.9%)	0.001	12.5 (3.9-39.5)
Vaginal pruritus	17 (53.1)	15 (46.9)	0.001	2.2 (1.4- 3.7)
HIV Infection	5 (35.7)	9 (64.3)	0.24	2.02 (0.61-6.6)
Trichomoniasis	3 (25.0)	9 (75.0%)	0.88	1.01(0.87-1.18)
Bacterial vaginosis	10 (35.7)	18 (64.3)	0.56	2.3 (0.91-5.9)
Chlamydia Cervicitis	3 (33.3)	6 (66.7)	0.46	1.7 (0.42-7.4)

In this study, Eleven (40.7%) of the women infected with vulvovaginal candidiasis were regular emergency oral contraceptive users. Acquisition of VVC was observed more in women who had multiple sexual partners in the preceding one month before the study, though not statistically significant ( $P = 0.068$ ). 96.3% (26 of 27) of women infected with VVC had multiple sexual partners. Douching was also found to increase the risk of acquisition of VVC. 25.9% of

women with VVC usually practice douching ( $P= 0.171$ ). (Table 6)

## DISCUSSION

Vulvovaginal candidiasis is a common gynecological health problem that is usually diagnosed in women of child bearing age;(24) and it has been associated with profound morbidity in all strata of women all over

the world.(11) In our study, the prevalence of vulvovaginal candidosis was 23.3% among women attending the family planning clinic in University College Hospital, Ibadan, Nigeria. This is the second most common infection after bacterial vaginosis (24.1%). The results of previous studies have confirmed similar rates that ranged from 18.5-30%. (25, 26) However, this rate is much lower than 84.5% discovered in Kano, Nigeria (27); 45.0% obtained by Namkinga et al in Dar es Salaam (28) and 40% found by Ibrahim and his colleagues in Maiduguri, Nigeria (29). This finding may not be unconnected with the fact that some of the women could have some underlying asymptomatic co-morbidity especially diabetes mellitus. The prevalence of yeast infections has also greatly increased worldwide because of indiscriminate use of antibiotics and immunosuppressive treatment.(30) Tarry et al have established that the use of oral contraceptives with high oestrogen content could encourage increased vaginal colonisation with *Candida* spp (31)

In this study, we found out that more than half (56.9%) of those seeking contraceptive use are in their active reproductive age (20-29 years old) and only 6.9% are in the 40-59 years age bracket. The mean age of women in this study is 28.7 years. This result is not unexpected as most of these young women were single, sexually active and prefer hormonal contraceptives. The finding is in concordance with previous study by Fisher and Boroditsky that examined sexual activity of single Canadian women aged 15-29.(32). Vulvovaginal candidiasis (VVC) was more common in women aged 20-29 years (59.3%) and lowest in 40-59 years (7.4%). This finding was also in agreement with other study that showed that the incidence of vulvovaginal candidiasis usually peaks in the third decade of life and reduces in women older than 40 years of age.(19) Despite the higher number of single women using hormonal contraceptives, prevalence of VVC was noticed more in married women living with their spouses. This might not be unconnected with increased sexual activities with married women. This finding agreed with Okungbowa et al who reported similar findings among married women.(19)

Sexual risk behaviours that have been documented to influence the acquisition of VVC were not statistically significant in our study. Early age of sexual debut (16-20 years) accounted for higher rate of VVC (16 of 27, 70.4%) but is not significantly associated with the infection. Women that engage with at least two or more casual sex partners also had increased rate of VVC (13 of 27, 48.1%) with no significant association for the acquisition of VVC. This was not in agreement with the findings of Hellberg et al and Rathod et al that reported age of sexual debut; casual sex partners

and regular oral sex being associated with repeated VVC. (33, 34) However, this is in agreement with the findings of Corsello et al that believed that increased number of sexual partners, age of sexual debut and increased frequency of sexual intercourse were not significantly risk factors associated with VVC.(35) Douching has been documented to be an important risk factor for the acquisition of vulvovaginal candidosis in previous studies (34, 36) .In our study, seven (25.9%) of the women who had VVC usually practice douching , however, this was not found to be statistically significant (p=0.171). Vaginal discharge was the commonest presentation (51 of 116, 44.0%) closely followed by vaginal pruritus (32 of 116, 27.6%). Some of these women also presented with the combination of vaginal discharge and vulvo-vaginal pruritus at the time of study. Almost all women that presented with vaginal pruritus (31 of 32, 96.9%) also had vaginal discharge. It was noticed that more than half of women (17 of 27, 53.1%) that presented with pruritus had VVC. Vaginal pruritus has been found to be significantly associated (p=0.001) with the occurrence of VVC in our study. These findings were in tandem with the results of similar studies that reported either combination of the symptoms or vaginal pruritus alone. (27, 37) (Table 6)

Types of hormonal contraceptives could also influence the acquisition of VVC. In this study, it was found that usages of oral and emergency contraceptives were found to be the commonest; however none of the hormonal contraceptive methods in this study was significantly associated with the acquisition of VVC. Eleven (22.9%) of the 48 women using emergency contraceptives had VVC and this is not statistically significant. This finding is similar to some studies that believed oral contraceptive alone may not influence the recurrence of VVC (16, 38). However, this is not in agreement with the findings of another study that found that users of oral contraceptive pills are at increased risk for acquisition of VVC.(6)

Vulvo-vaginal candidiasis has been linked as risk factor for the increased acquisition of HIV in hormonal contraceptive users. However, in our study there was no significant association between VVC and acquisition of HIV (P=0.24), only five (18.5%) of those women infected with VVC were seropositive for HIV infection. Our finding is not in agreement with the study of Martins et al that found a strong trend of association between the use of high dose of oral contraceptive pills and HIV acquisition. (5) The prevalence of Chlamydia cervicitis and gonococcal cervicitis in this study was 7.8% and 2.6% respectively. There was no significant association between the acquisitions of VVC and Chlamydial infection (P= 0. 46). This result was in tandem with

the previous studies regarding use of combined oral contraceptives and cervical Chlamydia infections (6, 39, 40). However, few of these studies reported conflicting results. In a prospective cohort study in the U.S. it was found that women using COCs were not at increased risk of developing gonorrhoeal or chlamydial cervical infection compared with women using non-hormonal contraception (39). Other infections like bacterial vaginosis, genital warts and trichomoniasis were not strongly associated with development of VVC in women using hormonal contraceptives in this study.

Young age, education, Christianity, non-marital status, and lower social class; influenced the choice and use of different hormonal contraceptive method though no significant association. Hormonal contraceptives users were associated with increased prevalence of vulvovaginal candidiasis in this study though not statistically significant. Vaginal discharge and vulvovaginal pruritus are the commonest

symptoms that are strongly associated with VVC. There is need for public health enlightenment programme to educate women using hormonal contraceptives methods to adopt safer sexual behaviours, as well as seeking early diagnosis and treatment of VVC and other sexually transmitted infections. The main limitation in this study was the lack of speciation of the non-albicans strains of *Candida* and was due to unavailability of Chromogenic Agar (CHROM agar) and other molecular diagnostic facilities in our center then. Temporal causality could not be established since this is a cross sectional study. Our small size limitation could also have interfered with the ability to fully explore the various associations.

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#### REFERENCES

1. Greydanus DE, Patel DR, Rimsza ME. Contraception in the adolescent: an update. *Pediatrics*. 2001;107(3):562-73.
2. Williamson LM, Parkes A, Wight D, Petticrew M, Hart GJ. Limits to modern contraceptive use among young women in developing countries: a systematic review of qualitative research. *Reproductive health*. 2009;6(1):3.
3. Riley HE, Steyn PS, Achilles SL, Bass E, Gray AL, Polis CB, et al. Hormonal contraceptive methods and HIV: research gaps and programmatic priorities. *Contraception*. 2017.
4. Vandale-Toney S, Conde-Gonzalez C. [Contraceptives, HIV, and other sexually transmitted diseases]. *Ginecologia y obstetricia de Mexico*. 1995;63:40-5.
5. Martin HL, Nyange PM, Richardson BA, Lavreys L, Mandaliya K, Jackson DJ, et al. Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1. *Journal of Infectious Diseases*. 1998;178(4):1053-9.
6. Baeten JM, Nyange PM, Richardson BA, Lavreys L, Chohan B, Martin HL, et al. Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. *American journal of obstetrics and gynecology*. 2001;185(2):380-5.
7. Krettek J, Arkin S, Chaisilwattana P, Monif G. Chlamydia trachomatis in patients who used oral contraceptives and had intermenstrual spotting. *Obstetrics and gynecology*. 1993;81(5 (Pt 1)):728-31.
8. Vandale-Toney S, Conde-Gonzalez CJ. [Contraceptives, HIV, and other sexually transmitted diseases]. *Ginecol Obstet Mex*. 1995;63:40-5.
9. Petitti DB. Combination estrogen-progestin oral contraceptives. *New England Journal of Medicine*. 2003;349(15):1443-50.
10. Burkman RT. Oral contraceptives: current status. *Clinical obstetrics and gynecology*. 2001;44(1):62-72.
11. Sobel JD. Vulvovaginal candidosis. *Lancet*. 2007;369(9577):1961-71.
12. Bakare R, Oni A, Umar U, Adewole IF, Shokunbi WA, Fayemiwo S, et al. Pattern of sexually transmitted diseases among commercial sex workers (CSWs) in Ibadan, Nigeria. *African journal of medicine and medical sciences*. 2002;31(3):243-7.
13. Novikova N, Mårdh PA. Characterization of women with a history of recurrent vulvovaginal candidosis. *Acta obstetrica et gynecologica Scandinavica*. 2002;81(11):1047-52.
14. Ekpenyong C, Inyang-Etoh E, Etebong E, Akpan U, Ibu J, Daniel N. Recurrent vulvovaginal candidosis among young women in south eastern Nigeria: the role of lifestyle and health-care practices. *International journal of STD & AIDS*. 2012;23(10):704-9.
15. Foxman B, Muraglia R, Dietz J-P, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *Journal of lower genital tract disease*. 2013;17(3):340-5.
16. Spinillo A, Capuzzo E, Nicola S, Baltaro F, Ferrari A, Monaco A. The impact of oral contraception on vulvovaginal candidiasis. *Contraception*. 1995;51(5):293-7.
17. Ahmad A, Khan AU. Prevalence of *Candida* species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. *European journal of obstetrics & gynecology and reproductive biology*. 2009;144(1):68-71.
18. Gupte P, Patil S, Pawaskar R. Vulvovaginal hygiene and care. *Indian Journal of Sexually Transmitted Diseases and AIDS*. 2009;30(2):130.

19. Okungbowa FI, Isikhuemen O, Dede AP. The distribution frequency of *Candida* species in the genitourinary tract among symptomatic individuals in Nigerian cities. *Revista iberoamericana de micologia*. 2003;20(2):60-3.
20. Cowan ST, Barrow G, Steel KJ, Feltham R. Cowan and Steel's manual for the identification of medical bacteria: Cambridge university press; 2004.
21. Lacey CJ, Lowndes CM, Shah KV. Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine*. 2006;24:S35-S41.
22. Ekweozor CC, Adeyemi-Doro FA, Ashiru JO, Osoba AO. Anogenital warts in patients attending the sexually transmitted diseases clinic in Ibadan, Nigeria. *African journal of medicine and medical sciences*. 1994;23(4):311-4.
23. Okesola AO, Fawole OI. Prevalence of human papilloma virus genital infections in sexually transmitted diseases clinic attendees in Ibadan. *West African journal of medicine*. 2000;19(3):195-9.
24. Aballéa S, Guelfucci F, Wagner J, Khemiri A, Dietz J-P, Sobel J, et al. Subjective health status and health-related quality of life among women with Recurrent Vulvovaginal Candidosis (RVVC) in Europe and the USA. Health and quality of life outcomes. 2013;11(1):169.
25. Otero L, Palacio V, Carreno F, Mendez F, Vazquez F. Vulvovaginal candidiasis in female sex workers. *International journal of STD & AIDS*. 1998;9(9):526-30.
26. Mirza N, Nsanze H, D'Costa L, Piot P. Microbiology of vaginal discharge in Nairobi, Kenya. *Sexually transmitted infections*. 1983;59(3):186-8.
27. Ugwa E. Vulvovaginal Candidiasis in Aminu Kano Teaching Hospital, North-West Nigeria: Hospital-Based Epidemiological Study. *Annals of medical and health sciences research*. 2015;5(4):274-8.
28. Namkinga L, Matee M, Kivaisi A, Moshiro C. Prevalence and risk factors for vaginal candidiasis among women seeking primary care for genital infections in Dar es Salaam, Tanzania. *East African medical journal*. 2005;82(3).
29. Ibrahim S, Bukar M, Mohammad Y, Audu B, Ibrahim H. Prevalence of vaginal candidiasis among pregnant women with abnormal vaginal discharge in Maiduguri. *Nigerian Journal of Medicine*. 2013;22(2):138-42.
30. Olowe O, Makanjuola O, Olowe R, Adekanle D. Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. *European Journal of Microbiology and Immunology*. 2014;4(4):193-7.
31. Tarry W, Fisher M, Shen S, Mawhinney M. *Candida albicans*: the estrogen target for vaginal colonization. *Journal of Surgical Research*. 2005;129(2):278-82.
32. Fisher WA, Boroditsky R, Bridges ML. The 1998 Canadian Contraception Study. *Canadian Journal of Human Sexuality*. 1999;8(3):161-216.
33. Hellberg D, Zdolsek B, Nilsson S, Mårdh P-A. Sexual behavior of women with repeated episodes of vulvovaginal candidiasis. *European journal of epidemiology*. 1995;11(5):575-9.
34. Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P. Epidemiologic features of Vulvovaginal Candidiasis among reproductive-age women in India. *Infectious diseases in obstetrics and gynecology*. 2012;2012.
35. Corsello S, Spinillo A, Osnengo G, Penna C, Guaschino S, Beltrame A, et al. An epidemiological survey of vulvovaginal candidiasis in Italy. *European journal of obstetrics & gynecology and reproductive biology*. 2003;110(1):66-72.
36. Heng LS, Yatsuya H, Morita S, Sakamoto J. Vaginal douching in Cambodian women: its prevalence and association with vaginal candidiasis. *Journal of epidemiology*. 2010;20(1):70-6.
37. Fardyazar Z, Habibzadeh S, Abdollahi-Fard S, Tello M. Vaginal azoles versus oral fluconazole in treatment of recurrent vulvovaginal candidiasis. *Archives of Clinical Infectious Diseases*. 2007;2(1).
38. Reed BD, Gorenflo DW, Gillespie BW, Pierson CL, Zazove P. Sexual behaviors and other risk factors for *Candida* vulvovaginitis. *Journal of women's health & gender-based medicine*. 2000;9(6):645-55.
39. Morrison CS, Sekadde-Kigondo C, Sinei SK, Weiner DH, Kwok C, Kokonya D. Is the intrauterine device appropriate contraception for HIV-1-infected women? *BJOG: an International Journal of Obstetrics & Gynaecology*. 2001;108(8):784-90.
40. Cottingham J, Hunter D. Chlamydia trachomatis and oral contraceptive use: a quantitative review. *Genitourinary medicine*. 1992;68(4):209-16.

## ORIGINAL ARTICLE

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### SCREENING AND PARTIAL PURIFICATION OF AMYLASE FROM ASPERGILLUS NIGER ISOLATED FROM DETERIORATED TOMATO (LYCOPERSICON ESCULENTUM MILL.) FRUITS

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#### ABSTRACT

Amylases (EC 3.2.1.1) are cellwall degrading enzymes associated with the pathogenicity of microorganisms in the spoilage of tomato fruits. The use of amylase in many industries has made it very important to optimize production process to achieve maximum yields. Screening and partial purification of Amylase from *Aspergillus niger* isolated from tomato (*Lycopersicon esculentum* Mill.) fruits was studied. Amylase producing fungi were isolated from fresh tomatoes kept at ambient temperature ( $28 \pm 1^\circ\text{C}$ ). Isolates were characterized on the basis of their morphological and cultural techniques. Partial purification of amylase was carried out by ammonium sulphate precipitation. The enzyme activity was determined and optimum conditions were obtained. The molecular weights of the crude and partially purified Amylase were determined by SDS PAGE method. A total of five isolates were obtained using basic screening technique for amylase activity, one of the isolates (Isolate code F2) exhibited maximum amylase activity. The fungi isolate code F2 was identified as *Aspergillus niger*. Optimum conditions for Amylase AMY F2 were ascertained at pH 6.0; temperature  $30^\circ\text{C}$ ; substrate concentration of 0.3mg/ml, and time of heating of less than 10min. The molecular weights of the crude and partially purified Amylase AMY F2 were found to be 55kDa and 35kDa respectively by SDS PAGE method. Microorganisms had been an encouraging means of economical production of enzymes in large scale for the food and drug industry.

Keywords: Amylase, Partial Purification, Enzyme, Tomato Fruits

### RENDEMENT ET PURIFICATION PARTIELLE DE L'AMYLASE DE ASPERGILLUS NIGER ISOLÉ À PARTIR DE TOMATE DÉTERIORÉ (LYCOPERSICON ESCULENTUM MILL.) FRUITS

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#### ABSTRAIT

Les amylases (EC 3.2.1.1) sont des enzymes dégradant les parois cellulaires associées à la pathogénicité des microorganismes dans la détérioration des fruits à la tomate. L'utilisation de l'amylase dans de nombreuses industries a rendu très important d'optimiser le processus de production pour obtenir des rendements maximaux. Le dépistage et la purification partielle de l'amylase d'*Aspergillus niger* isolés à partir des fruits à la tomate (*Lycopersicon esculentum* Mill.) ont été étudiés. Les champignons producteurs d'amylase ont été isolés à partir de tomates fraîches conservées à température ambiante ( $28 \pm 1^\circ\text{C}$ ). Les isolats ont été caractérisés en fonction de leurs techniques morphologiques et culturelles. La purification partielle de l'amylase a été réalisée par précipitation au sulfate d'ammonium. L'activité enzymatique a été déterminée et des conditions optimales ont été obtenues. Les poids moléculaires de l'amylase brut et partiellement purifiée ont été déterminés par un procédé SDS PAGE. Au total, cinq isolats ont été obtenus en utilisant une technique de dépistage basique pour l'activité amylase, l'un des isolats (code isolé F2) présentait une activité amylase maximale. Le code isolant F2 des Fusions a été identifié comme *Aspergillus niger*. Les conditions optimales pour Amylase AMY F2 ont été déterminées à pH 6,0; température  $30^\circ\text{C}$ ; concentration de substrat de 0,3 mg / ml et temps de chauffage de moins de 10 min. On a trouvé que les poids moléculaires de l'amylase brute et partiellement purifiée étaient respectivement de 55 kDa et 35 kDa par le procédé SDS PAGE. Les microorganismes ont été un moyen encourageant de production économique d'enzymes à grande échelle pour l'industrie alimentaire et pharmaceutique.

Mots-clés: Amylase, Purification partielle, Enzyme, Fruits tomates

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## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) has fleshy endocarp which belongs to the berry class (1). They are rich in nutrients such as vitamins, minerals, dietary fiber and protein (2). Tomato fruits have 95% water and 4.5% carbohydrate (3). In view of these, the tomato fruit is often attacked by microorganisms most especially during and after harvest, which makes tomato spoilage to occur often (4). Microorganisms which are related with the deterioration of tomato fruits can attack them after harvesting; or during the process of storage and distribution (5). The spoilage usually occurs on the fruits after a period of time (6). The plant cell wall which is a protective layer that coats the outer surface usually plays many roles, including controlling movement of water into the fruit (7). It also filters potentially damaging UV light, and limits attack by pathogens (5). In Nigeria, tomatoes are kept at the open markets; the fruits are often displayed in baskets and on benches for the prospective customers, thereby exposing them to opportunistic microbial infections especially mycotoxins (8). Post-harvest Infections in tomato fruits could occur during storage, transportation, packaging and distribution (loading and offloading) at various point of sale at which bacteria and fungi are present (9). Adequate knowledge and careful handling procedure of the tomatoes can reduce wastage of the fruits (10). Previous study on deterioration of tomatoes by microorganism showed that post-harvest damages are mostly due to attack by fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* of up to 90% prevalence (7,11). Thus, there is need for constant research to isolate the fungi associated with tomato fruits deterioration with the view to providing suitable solutions of preserving the tomato fruits, keeping them fresh up to when they reach the consumers (12). This helps to ensure all year round availability and to protect the public health (13). Contamination of fresh tomatoes by microorganisms occurs naturally because the fruits are exposed to the environments during harvest, transportation and storage (7). Hence, the tomato normal flora and pathogen contamination often occurs at different points (14). Tomato micro flora which are mostly fungi found on fresh tomato fruits are the yeasts and molds (8). Microorganisms are present everywhere and around us (15). Pathogens such *Escherichia coli* and *Listeria monocytogenes* may be found on the cell wall of the fresh tomato fruits and this are of public health impact (7). *Enterobacteriaceae* and Gram negative bacteria of the genus *Pseudomonas* may also be found the surface of tomato fruits. Yeast of the genus *Saccharomyces* populations of  $10^5$ - $10^6$  cfu/ml had been reported (8, 16). Bacterial populations

ranging from  $10^4$  -  $10^9$  cfu/ml and mold from the genus *Aspergillus* and *Rhizopus* with population ranging from  $10^4$  -  $10^9$  spores/ml in fresh tomatoes had also been reported (5). Growth of pathogenic microorganisms on the intact surface of fresh tomato is not common as the microbes cannot produce cell wall degrading enzymes until they gain entrance into the tomatoes where they can get nutrients and water (17). Damage to the cell wall of the tomato fruits therefore helps the food borne pathogens to grow and multiply, most especially under room temperatures (7). Refrigerator temperature arrests the growth of microorganisms while those that could survive extremely low temperatures still grow when the cell walls are broken due to the fact that released fluids serve as nutrients for the microorganisms (8,18).

Fungi such as *Aspergillus niger* can produce different cell wall degrading enzymes that breaks down large polysaccharides into simple reducing sugars which are used up for growth and multiplication (19). These enzymes have a lot of industrial and environmental importance. For example, they are used in brewery, food processing and bioremediation of organic pollutants (19,20). **Amylases (EC 3.2.1.1) are extracellular enzymes which catalyzes starch hydrolysis (21). The enzyme catalyzes the breakdown of  $\alpha$ -1,4-O-glycosidic bonds in polysaccharides by leaving  $\alpha$ -anomeric configuration in the resulting compounds (22). The amylase family exists in two forms as either starch modifying enzymes or starch hydrolyzing enzymes (7). Amylases can be recovered from different sources of plants, animals and microorganisms origin (23). Some amylases require calcium ions ( $Ca^{2+}$ ) for their optimum activity and structural stability (21). Amylases have a wide range of industrial application which ranges from production of dextrins, sugar syrup and sweeteners (23). They are usually imported because of their application in the breweries, bakery and factories making soaps and detergents (24). Amylase makes up to 40% of enzyme production in the world (23). However, prolonged storage of amylases often results in reduced activity (21).**

## MATERIALS AND METHODS

One hundred and eighty (180) fresh tomato fruits were purchased from the Ota market, Ota, Ogun State, Nigeria. They were sorted, washed and transported to the microbiology laboratory of the Department of Biological Sciences, Covenant University, Ota, Ogun State in sterile polythene bags. Sixty (60) samples designated as P were stored in a locally made post-harvest storage system, the second set of sixty (60) fruits designated R were stored in the refrigerator and

**the last set of sixty (60) fruits designated C were stored at ambient temperature in the laboratory. All samples were analyzed and sampling was done for a period of fourteen days.**

#### **Isolation of microorganisms from the tomato fruit samples**

The total spore count was determined by pour plate method, each tomato fruit samples was diluted using sterile distilled water. One (1) milliliter of the dilution of  $10^4$  was plated onto Sabroaud Dextrose Agar (SDA) and incubated at room temperature for 3-5 days for the fungal population.. **Fungal samples were collected from the center of the growth covering the plates with the aid of sterile inoculating needle. The samples were smeared on glass slides and stained with lactophenol cotton blue (9).**

#### **Identification of the fungal isolates**

The fungi isolated in this research were identified using identification methods contained in the illustrated Handbook of Fungi (25). The colony color, pattern of growth, and sporulation style were observed (6). Microscopic observation was carried out on the mature sporulating growths five days after inoculation on SDA plates. Morphological characteristics like arrangement and shape of spores, type of sporangia, and type of hyphae, presence or absence of septa on hyphae were examined under the high power objective of a compound binocular microscope (26). Microscopic examination was carried out after Gram staining the Yeast isolates, while Lactophenol blue staining was carried out on the remaining fungal isolates which are molds (27).

#### **Preparation of fungal spore suspension for production of enzymes**

A spore suspension containing 15ml of sterile distilled water and three drops of tween 80 solution was used to obtain the spore from a 120 h-old culture of each fungal isolate. The suspension was centrifuged at 4000rpm for 6 minutes. The supernatant was discarded and replaced with 10ml of sterile distilled water. The spore suspension was diluted serially from  $10^{-1}$  to  $10^{-7}$ . The fungal spores were counted using the Neubauer Counting Chamber according to the method of (28).

#### **Growth of Isolated Fungi in Basal Salt Medium Containing Starch**

The Basal Salt Medium, for the growth of the isolated fungi and the enzyme production, was prepared according to the method of (29) containing (in one liter):  $\text{KH}_2\text{PO}_4$  - 2.0g,  $(\text{NH}_4)_2\text{SO}_4$  - 1.4g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.3g,  $\text{CaCl}_2$  - 0.3g, Urea - 0.3g, Tween 80 - 1ml, Yeast extract - 0.4g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  - 5mg,  $\text{MnSO}_4$  - 1.6mg,

$\text{ZnSO}_4$  - 1.4mg,  $\text{CoCl}_2$  - 2.0mg. The medium was supplemented with 1% (w/v) soluble starch. One hundred milliliters of growth medium was inoculated with 1 ml of an aqueous spore suspension containing approximately  $5 \times 10^6$  spores/ml,  $8 \times 10^6$  spores/ml and  $10 \times 10^6$  spores/ml of isolated fungi F1, F2 and F3 respectively. Experimental flasks contained the inoculated sterilized medium while control flasks contained only the sterilized medium. Both experimental and control flasks were incubated without shaking according to the method of (30) for eight days at room temperature ( $25^\circ\text{C}$ ).

#### **Crude Enzyme Extraction by Filtration**

To separate the liquid enzyme mixture and the mycelia mat along with the spore bodies' filtration technique was employed. The mixture was filtered through Whatman No. 1 filter paper and then centrifuged at 5,000 rpm for 30 minutes according to the method of (31). This served as the crude enzyme.

#### **Amylase Assay**

Amylase activity was determined using the method described by (21) whereby the reaction mixture consisted of 2 ml of 0.2% (w/v) starch in 0.2M citrate phosphate buffer at pH 6.0 as substrate and 0.5 ml of enzyme. The control experiments consisted of only 2 ml of the prepared substrate. The content of the experimental and control test tubes were incubated at  $35^\circ\text{C}$  for 20 minutes. The reaction in each test tube was terminated with 3 ml of 1N Hydrochloric acid. The enzyme (0.5 ml) was then added to the control tube. Two milliliters of the mixture from each of the set of experimental and control was transferred into new set of clean test tube. 3ml of 0.1N Hydrochloric acid was added into the content of each new test tubes after which 0.1 ml of iodine solution was added. The optical density reading was taken at 670nm. One unit of enzyme activity was defined as the amount of enzyme which produced 0.1 percent reduction in the intensity of the blue color of starch - iodine complex under conditions of the assay. Specific activity was calculated as enzyme units per milligram protein.

#### **Ammonium sulphate precipitation**

Ammonium sulphate precipitation was done according to the method described by (31). Solid Ammonium sulphate of analytical grade was added to crude enzyme preparation to 90% saturation. The solution was then kept at  $4^\circ\text{C}$  for 24h. After 24 h, the precipitate was removed by centrifuging at 5000rpm for 30 minutes, the supernatant was decanted and the precipitate was then dissolved again in 1ml of 0.2M citrate phosphate buffer (pH 6.0). The enzyme solution was dialyzed overnight against five changes of the similar buffer. Dialysis was done in acetylated cellophane tubing prepared from Visking dialysis

tubing (Gallenkamp) as described by (32). The protein content of the dialysate was determined by the method of (33,34). Enzyme assay of the dialysate was also determined as described above.

#### Effect of Temperature on Amylase AMY F2

To determine the optimum temperature for the amylase production, the partially purified enzyme were subjected to various temperatures; 20°C, 25°C, 30°C, 35°C, 40°C and 45°C for 20 min. The enzyme activity was determined as previously described by (21)

#### Effect of pH on Amylase AMY F2

The effect of pH on the enzymes activity was determined by varying the pH of the substrate from 4.5 to pH 8. The substrate consisted of 0.2% (w/v) of soluble starch dissolved in 0.2M citrate phosphate buffer pH 6.0 of varying pH. Incubation was at 35°C for 20 min. Amylase activity was determined as previously described by (23).

#### Effect of Substrate Concentrations on Amylase AMY F2

The effect of various substrate concentrations was determined using various concentrations of starch in 0.2M citrate phosphate buffer; 0.05%, 0.1%, 0.15%, 0.2%, and 0.25%. The reaction mixture contained 1ml of starch and 0.5ml of enzyme incubated at 35°C for 20 min. Amylase activity was determined as previously described by (30).

#### Effect of Time of Heating on Stability of Amylase AMY F2

The effect of time of heating on the stability of Amylase enzyme was determined. Samples of partially purified enzyme was heated at 80°C for different periods of time (0, 5, 10, 15, 20, 25, 30 min) respectively. The reaction mixtures consisted of 1 ml of starch and 0.5ml of the enzyme solutions. Amylase activity was determined as previously described by (21).

#### Molecular weight determination of Amylase AMY F2

The crude enzyme and ammonium sulphate precipitated amylase sample were loaded on SDS-PAGE and a protein profile was obtained according to the method of (35). The molecular weight of the enzyme was determined by comparing them with the molecular weight marker. The ammonium sulphate precipitated sample was run on native PAGE. The native gel placed over the Starch agar gel was then subjected to zymogram staining as described by (35).

## RESULTS

#### Enumeration of Fungal Population

The total fungal spore count for the tomato fruits stored in the post-harvest storage system was within the range of  $1.0 \times 10^5$  -  $4.0 \times 10^6$  spores/ml while those fruits stored in the refrigerator had fungal spore count range of  $1.0 \times 10^5$  -  $3.0 \times 10^6$  spores/ml and fungal spore count of  $2.0 \times 10^2$  -  $4.0 \times 10^{11}$  spores/ml for the fruits stored at ambient temperature (Table 1).

TABLE 1: ENUMERATION OF FUNGAL POPULATION

Days	Post-Harvest Storage System (P) spores/ml	Refrigerator (R) spores/ml	Ambient temperature (C) spores/ml
2	$1.0 \times 10^5$	$1.0 \times 10^5$	$1.2 \times 10^5$
3	$3.0 \times 10^5$	$4.0 \times 10^5$	$2.0 \times 10^5$
4	$3.0 \times 10^5$	$2.0 \times 10^5$	$4.0 \times 10^5$
5	$5.0 \times 10^5$	$1.0 \times 10^5$	$1.0 \times 10^6$
6	$3.0 \times 10^6$	$4.0 \times 10^5$	$2.0 \times 10^9$
7	$4.0 \times 10^6$	$2.0 \times 10^6$	$2.0 \times 10^9$
8	$3.0 \times 10^6$	$3.0 \times 10^6$	$5.0 \times 10^9$
9	$4.0 \times 10^6$	$2.0 \times 10^6$	$1.0 \times 10^{11}$
10	$4.0 \times 10^6$	$1.0 \times 10^6$	$2.0 \times 10^6$
11	$4.0 \times 10^6$	$1.0 \times 10^6$	$1.0 \times 10^6$
12	$2.0 \times 10^6$	$1.0 \times 10^6$	$2.0 \times 10^6$
13	$1.0 \times 10^6$	NG	$4.0 \times 10^6$
14	$1.0 \times 10^6$	NG	$2.0 \times 10^2$

KEY: NG - No growth

### Identification of Fungal Isolates

The result revealed five pure fungal isolates from tomato fruits (*Lycopersicon esculentum* Mill.). Gram reaction result revealed presence of yeast (*Saccharomyces cerevisiae*) while the molds were identified to be *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, and *Aspergillus fumigatus* (Table 2).

### Production of Amylase

The amylase produced by fungal isolate code F2 obtained from tomato fruits stored at the ambient temperature on day 7 produced amylase with a total activity of 0.266 units/ml, protein content of 0.338 mg/ml and a specific activity 0.787 units/mg proteins and a yield of 63% after ammonium sulphate precipitation (Table 3).

TABLE 2: IDENTIFICATION OF THE FUNGAL ISOLATES

Isolate code	Colony Colour	Nature of hyphae	Asexual spore	Somatic structure	Identity of Fungi Isolates
F1	Deep green colony	Septate	Globose conidia	Filamentous	<i>Aspergillus flavus</i>
F2	Blackish colony	Septate	Globose conidia	Filamentous	<i>Aspergillus niger</i>
F3	Cotton white	Non septate	Sporangiospore	Filamentous	<i>Rhizopus stolonifer</i>
F4	Light green colony	Septate	Globose conidia	Filamentous	<i>Aspergillus fumigatus</i>
F5	Shiny milky colony (moist)	No hyphae	Budding cells	Unicellular	<i>Saccharomyces cerevisiae</i>

TABLE 3: PARTIAL PURIFICATION OF AMYLASE OBTAINED FROM MICROORGANISMS ISOLATED FROM TOMATO (*Lycopersicon esculentum* MILL.) FRUITS

Enzyme code	Protein step	Total (units/ml)	Activity	Protein (mg/ml)	Specific activity (units/mg)	Yield (%)	Purification fold
AMY F2	Crude extract	0.27		0.34	0.79	100.00	1.00
	Ammonium sulphate precipitation	0.17		0.02	9.33	63.00	1.86

### Characterization of Enzymes

#### Effect of Temperature on Amylase AMY F2

The temperature of incubation affected the amylase activity tremendously. The activity of amylase AMY F2 increased with an increase in incubation temperature until an optimum was reached (Figure 1). Subsequent increase in temperature beyond the optimum temperature led to reduction in the enzyme activity. The optimum temperature for amylase AMY F2 was 30°C.

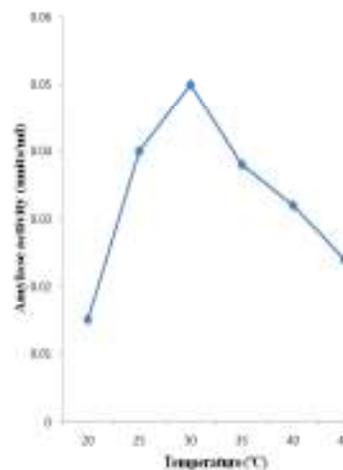
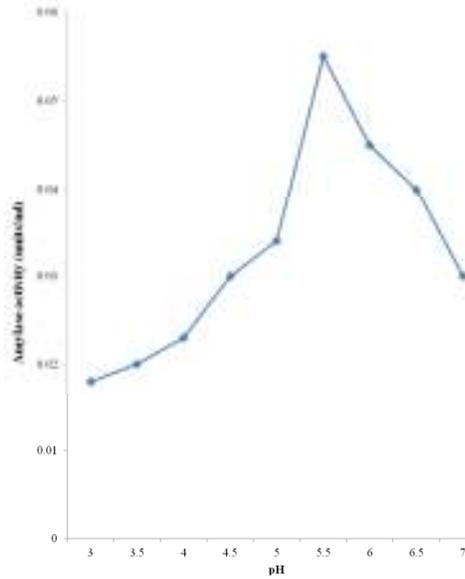


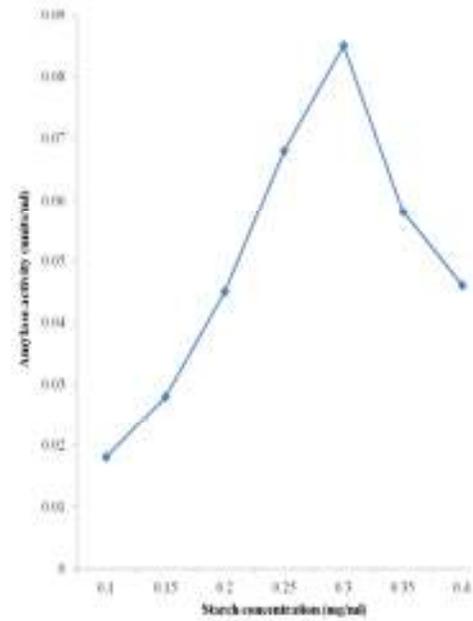
FIGURE 1: EFFECT OF TEMPERATURE ON PARTIALLY PURIFIED AMYLASE AMY F2

#### Effect of pH on Amylase AMY F2

The pH of the reaction mixtures had effect on the activities of the amylase produced both by the fungi isolate F2. The enzyme activities increased as the pH increases and also decreased when the optimum pH value was reached. The optimum pH for amylase AMY F2 was at pH 6.0 (Figure 2).



**FIGURE 2: EFFECT OF PH ON PARTIALLY PURIFIED AMYLASE AMY F2**



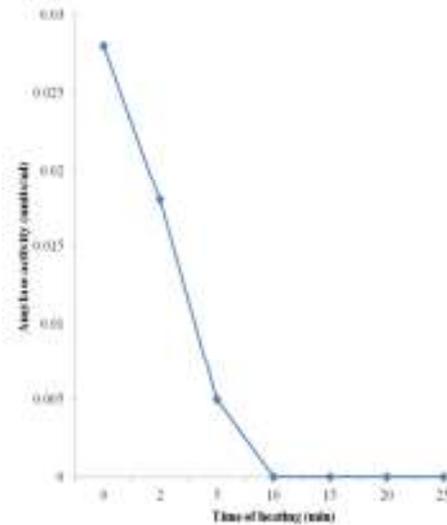
**FIGURE 3: EFFECT OF SUBSTRATE CONCENTRATION ON PARTIALLY PURIFIED AMYLASE AMY F2**

**Effect of Substrate Concentration on Amylase AMY F2**

The activity of the amylase produced by fungi F2 isolated from tomato fruits increases with an increase in concentration of the substrate. This continued to increase until an optimum concentration of substrate was attained (Figure 3). The optimum substrate concentration of AMY F2 was 0.30 mg/ml.

**Effect of Heat on Amylase AMY F2**

The activity of amylase on heating at 80°C decreased with an increase in the time of heating. When the amylase was subjected to heat for 2 minutes, activities of approximately was lost. Amylase AMY F2 was completely inactivated after 10 minutes of heating (Figure 4).



**FIG. 4: EFFECT OF HEAT (80°C) ON PARTIALLY PURIFIED AMYLASE AMY F2**

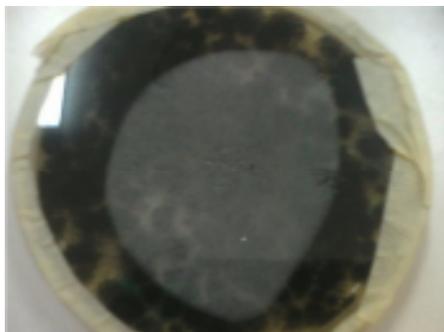


PLATE 1: ASPERGILLUS NIGER ISOLATED FROM TOMATO (LYCOPERSICON ESCULENTUM MILL.) FRUITS

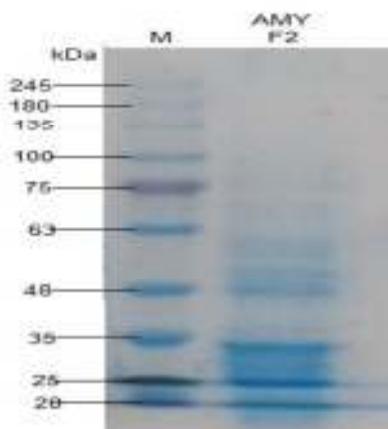


PLATE 2: SDS PAGE OF THE CRUDE AMYLASE AMY F2

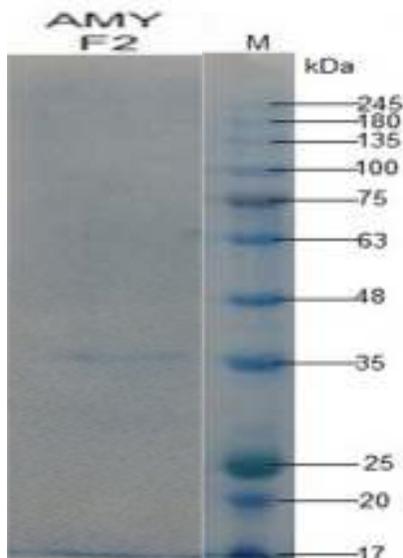


PLATE 3: SDS PAGE OF THE PARTIALLY PURIFIED AMYLASE AMY F2

### Molecular Weight Determination of Amylase AMY F2

Molecular weight of the crude Amylase and partially purified Amylase AMY F2 are 55KDa and 35KDa respectively (Plate 2 and 3).

### DISCUSSION

The result of this research had the total fungal population ranges between  $1.0 \times 10^5$  and  $4.0 \times 10^6$  spores/ml for the tomato fruits stored in the post-harvest storage system, those in the Refrigerator ranges between  $1.0 \times 10^5$  and  $3.0 \times 10^6$  spores/ml while the tomato fruits stored at ambient temperatures had fungal population range of  $2.0 \times 10^2$  and  $4.0 \times 10^{11}$  spores/ml. This results is similar to the finding of (36) having an average fungal counts ranging between  $1.3 \times 10^3$  and  $2.0 \times 10^3$  spores/ml and identified them to be *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium oxysporium*, *Saccharomyces cerevisiae*, *Alternaria alternate*, *Penicillium digitatum* and *Geotrichum candidum*. (37) also reported an average fungal count of  $5.4 \times 10^5$  spores/ml and  $2.0 \times 10^3$  spores/ml for *Penicillium notatum* and *Aspergillus flavus* at ambient temperature for tomato samples from new Benin Market in Edo State, Nigeria.

The fungi isolated from the tomato fruits were members of the yeast family *Saccharomyces cerevisiae* and the mold family *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer*. (21) reported *Aspergillus* sp. and *Saccharomyces cerevisiae* as the fungi associated with spoilage of tomato fruits. (38) also reported two fungi, *Aspergillus flavus* and *Rhizopus stolonifer* after five days of storage of fresh tomatoes at ambient temperatures.

The result of this research revealed the ability of some fungal isolates to produce Amylase. (31) and (39) reported production of amylase from fresh tomato fruits. The production from fungi and yeasts has also been reported by (21). The production of amylase from microorganisms isolated from other sources were also reported from other fruits and vegetables (40), bread (28), Irish potato (30) and even soil (41).

Members of the genera *Aspergillus*, *Rhizopus* and *Saccharomyces* were reported by (7,42,43) for their potentials to secrete a number of cell wall degrading enzymes.

The optimum temperature of amylase produced in this study was 30°C. This is in support of the reports of (23,39). (28) had earlier reported that the optimum temperature for amylase production by *Penicillium citrinum* ranges from 30°C - 35°C depending on source. However, this report contrasts with earlier

reports by (23) and (44) which reported that amylase production by was optimum at 37°C. Optimum pH was identified as 6.0 for Amylase AMY F2. This result confirmed that of previous researchers that who reported that amylase was most active between pH 5.0 and 6.0 (43). The optimum substrate concentration for the starch was 0.25mg/ml. (23) reported optimum substrate concentration of 0.20mg/ml for amylase. In this research, the effect of time of heating was investigated at 80°C over a period of 30min revealed loss of enzyme activity within 10min of heating. This confirmed earlier reports by (23,28,45). (30) revealed that amylase was active for over 5min of heating at 80°C. There was continuous reduction in the enzyme activity as heat was applied. After 10min of heating, enzyme activity was completely lost in all enzymes as was also revealed by (21) .

## REFERENCES

1. Esua OJ, Chin NL, Yusof YA, Sukor R. Postharvest deterioration of tomato ( *Solanum lycopersicum* ) and associated effects on antioxidant bioactive contents. Acta Hort [Internet]. 2017 Mar [cited 2017 Sep 7];(1152):327-34. Available from: [http://www.actahort.org/books/1152/1152\\_44.htm](http://www.actahort.org/books/1152/1152_44.htm)
2. Ilupeju EAO, Akanbi WB, Olaniyi JO, Lawal BA, Ojo MA, Akintokun PO. Impact of organic and inorganic fertilizers on growth, fruit yield, nutritional and lycopene contents of three varieties of tomato (*Lycopersicon esculentum* (L.) Mill) in Ogbomoso, Nigeria. African J Biotechnol. 2015;14(31):2424-33.
3. Chika E, Ifeanyichukwu I, Carissa D, Thomas A, Okoro O, Joshua E, et al. Occurrence of Metallo-Beta-Lactamase-Producing Enterobacteriaceae from a Local Poultry Farm in Abakaliki, Nigeria. Int J Appl Pharm Sci Res [Internet]. 2016 Jun 12 [cited 2017 Sep 8];1(2):70-5. Available from: <http://www.sierrajournals.com/direct/index.php/IJAPSR/article/view/17>
4. Agbabiaka TO. Microbial Deterioration of Tomato Fruit ( *Lycopersicon esculentum* ) Sold in Three Popular Markets in Ilorin, Kwara State, Nigeria. Fountain J Nat Appl Sci [Internet]. 2015 [cited 2017 Sep 7];4(1). Available from: <http://fountainjournals.com/index.php/fujnas/article/view/54>
5. Udoh IP, Eleazar CI, Ogeneh BO, Ohanu ME.

This research work recommends that tomato fruits could be potential source of amylase enzyme needed for various industrial processes. The Potential use of amylase has made it very important to optimize production process to achieve maximum yields.

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Studies on Fungi Responsible for the Spoilage / Deterioration of Some Edible Fruits and Vegetables. Adv Microbiol. 2015;5(April):285-90.

6. Amadi JE, Nwaokike P, Olan GS, Garuba T. ISOLATION AND IDENTIFICATION OF FUNGI INVOLVED IN THE POST-HARVEST SPOILAGE OF GUAVA (*Psidium guajava*) IN AWKA METROPOLIS. 2014 [cited 2017 Sep 7];4(10). Available from: <http://eaas-journal.org/survey/userfiles/files/v4i1002 Biological Engineering.pdf>
7. Al-Hindi RR, Al-Najada AR, Mohamed SA. Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. African J Microbiol Res. 2011;5(4):443-8.
8. Chinedu SM, Emmanuel E. Original Research Article Isolation of Microorganisms associated with Deterioration of Tomato ( *Lycopersicon esculentum* ) and Pawpaw ( *Carica papaya* ) Fruits. Int J Curr Microbiol Appl Sci. 2014;3(5):501-12.
9. Firas AA, Brent SS, Anne MA. Postharvest diseases of tomato and natural products for disease management. African J Agric Res [Internet]. 2017 Mar 2 [cited 2017 Sep 7];12(9):684-91. Available from: <http://academicjournals.org/journal/AJAR/article-abstract/B78AB8662982>
10. Schneider KR, De J, Li Y, Sreedharan A, Goodrich Schneider R, Danyluk MD, et al. Microbial evaluation of pre- and post-

- processed tomatoes from Florida, New Jersey and Maryland packinghouses. Food Control [Internet]. 2017 [cited 2017 Sep 22];73:511-7. Available from: <http://www.sciencedirect.com/science/article/pii/S0956713516304881>
11. Chu EH, Shin EJ, Park HJ, Jeong RD. Effect of gamma irradiation and its convergent treatment for control of postharvest Botrytis cinerea of cut roses. Radiat Phys Chem. 2015;
  12. Zoellner C, Venegas F, Churey JJ, Dávila-Aviña J, Grohn YT, García S, et al. Microbial dynamics of indicator microorganisms on fresh tomatoes in the supply chain from Mexico to the USA. Int J Food Microbiol [Internet]. 2016 Dec [cited 2017 Sep 7];238:202-7. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0168160516304858>
  13. Ogundipe FO, Bamidele FA, Adebayo-Oyetoro AO, Ogundipe OO, Tajudeen OK. Incidence of Bacteria with Potential Public Health Implications in Raw Lycopersicon esculentum (Tomato) Sold in Lagos State, Nigeria. Niger Food J. 2012;30(2):106-13.
  14. Schneider KR, De J, Li Y, Sreedharan A, Goodrich Schneider R, Danyluk MD, et al. Microbial evaluation of pre- and post-processed tomatoes from Florida, New Jersey and Maryland packinghouses. Food Control [Internet]. 2017 [cited 2017 Sep 7];73:511-7. Available from: <http://www.sciencedirect.com/science/article/pii/S0956713516304881>
  15. Valdramidis V. Predictive microbiology. In: Modeling in Food Microbiology: From Predictive Microbiology to Exposure Assessment. 2016.
  16. Agriculturae S, Bello OB, Olawuyi OJ, Azeez AH, Adebisi OS, Owoade TA. Microorganisms causing post harvest tomato (solanum lycopersicum l.) fruit decay in nigeria. 2016 [cited 2017 Sep 22];13(2016):93-6. Available from: <http://pscpub.com/Journals/Data/JList/Scientia Agriculturae/2016/Volume 13/Issue 2/5.pdf>
  17. Olasehinde GI, Okolie ZV, Oniha MI, Adekeye BT, Ajayi AA. In vitro antibacterial and antifungal activities of Chrysophyllum albidum and Diospyros monbuttensis leaves. J Pharmacogn Phyther [Internet]. 2016 Jan 31 [cited 2017 Sep 7];8(1):1-7. Available from: <http://academicjournals.org/journal/JPP/article-abstract/98A32D856891>
  18. Larsen MH, Koch AG, Ingmer H. *Listeria monocytogenes* efficiently invades Caco-2 cells after low-temperature storage in broth and on deli meat. Foodborne Pathog Dis. 2010;
  19. Ajayi, AA, Adedeji OM . Modification of Cell Wall Degrading Enzymes from Soursop (*Annona muricata*) Fruit Deterioration for Improved Commercial Development of Clarified Soursop Juice (A Review). Med Aromat Plants. 2014;4(1):1-5.
  20. Koyani RD, Rajput KS. Journal of Environmental Science , Computer Science and Engineering & Technology Fungal Enzymes : Dynamic Strategy and Significance for Remediating Chlorinated Compounds. 2016;5(1):10-30.
  21. Adejuwon AO, Adejuwon MA, Ajayi AA, Bamkefa BA, Omololu-Aso J, Alao OO, et al. Effect of some nitrogen sources of growth medium on  $\alpha$ -amylase production by *Penicillium solitum* and *Aspergillus rubrum* isolated from yam (*Dioscorea alata*). Researcher [Internet]. 2013 [cited 2017 Sep 7];5(2). Available from: <http://www.sciencepub.net/researcher>
  22. Mari M, Bautista-Baños S, Sivakumar D. Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. Postharvest Biol Technol [Internet]. 2016 Dec [cited 2017 Sep 7];122:70-81. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0925521416300734>
  23. Matpan Bekler F, Güven K. Isolation and production of thermostable  $\alpha$ -amylase from thermophilic *Anoxybacillus* sp. KP1 from Diyadin hot spring in Ağrı, Turkey. Biologia (Bratisl). 2014;
  24. Awojobi KO, Adejuwon MA, Ologbosere O. 7 1\*, 2, 3. 2013;5(6):10-7.
  25. Reddy PL, Sreeramulu A. Isolation, identification and screening of pectinolytic fungi from different soil samples of Chittoor District. Int J Life Sci Biotechnol Pharma Res. 2012;1(3):186-93.

26. Vandewoestyne M, Goossens K, Burvenich C, Van Soom A, Peelman L, Deforce D. Laser capture microdissection: should an ultraviolet or infrared laser be used? *Anal Biochem* [Internet]. 2013 Aug 15 [cited 2017 Sep 22];439(2):88-98. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23643622>
27. Xu A, Pahl DM, Buchanan RL, Micallef S a. Comparing the Microbiological Status of Pre- and Postharvest Produce from Small Organic Production. *J Food Prot*. 2015;
28. Adejuwon AO, Oluduro AO, Agboola FK, Olutiola PO, Segal SJ. Expression and characterization of  $\alpha$ -Amylases from *penicillium citrinum* with bread as growth substrate. 2015;9(August):2136-51.
29. Sternberg D. A method for increasing cellulase production by *Trichoderma viride*. *Biotechnol Bioeng* [Internet]. 1976 Dec [cited 2017 Sep 23];18(12):1751-60. Available from: <http://doi.wiley.com/10.1002/bit.260181209>
30. Adejuwon AO, Ladokun OA. Effect of Carbon Source of Growth on  $\alpha$ -Amylase Production by a Strain of *Penicillium funiculosum* Thom. Isolated from Irish Potato (*Solanum tuberosum* Linn.); Comparative Studies 1. Introduction  $\alpha$ -Amylases are starch degrading enzymes that catalyze th. 2015;3(1):1-11.
31. Adedeji OM AA. Modification of Cell Wall Degrading Enzymes from Soursop (*Annona muricata*) Fruit Deterioration for Improved Commercial Development of Clarified Soursop Juice (A Review). *Med Aromat Plants* [Internet]. 2014;4(1):1-5. Available from: <http://www.omicsgroup.org/journals/modification-of-cell-wall-degrading-enzymes-from-soursop-annona-muricata-fruit-deterioration-for-improved-commercial-development-of-clarified-soursop-juice-a-review-2167-0412.1000178.php?aid=40924>
32. WHITAKER DR, HANSON KR, DATTA PK. Improved procedures for preparation and characterization of *myrothecium cellulase*. 2. Purification procedures. *Can J Biochem Physiol* [Internet]. 1963 Mar [cited 2017 Sep 23];41:671-96. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/14000266>
33. Gebauer W. Protein Quantitation: Lowry Protocol. In: *Encyclopedia of Life Sciences* [Internet]. Chichester: John Wiley & Sons, Ltd; 2003 [cited 2017 Sep 23]. Available from: <http://doi.wiley.com/10.1038/npg.els.0003754>
34. PROTEINS (LOWRY) PROTOCOL. [cited 2017 Sep 23]; Available from: <http://web.itu.edu.tr/~dulekgurgen/Proteins.pdf>
35. Gopinath SM, Shareef I, Ranjit S. Isolation, Screening and Purification of Cellulase from Cellulase Producing *Klebsiella variicola*. *Int J Sci Res*. 2014;3(6):1398-403.
36. Onuorah and Orji (2015). Onuorah and Orji (2015) - Google Search [Internet]. [cited 2017 Sep 23]. Available from: [https://www.google.com/search?q=Onuorah+and+Orji+%282015%29+&oq=Onuorah+and+Orji+%282015%29+&gs\\_l=psy-ab.3...6710.10724.0.13375.31.3.0.0.0.1476.1476.7-1.2.0...0...1.1.64.psy-ab..29.0.0.0...1969.PmBG\\_b0d7ac](https://www.google.com/search?q=Onuorah+and+Orji+%282015%29+&oq=Onuorah+and+Orji+%282015%29+&gs_l=psy-ab.3...6710.10724.0.13375.31.3.0.0.0.1476.1476.7-1.2.0...0...1.1.64.psy-ab..29.0.0.0...1969.PmBG_b0d7ac)
37. Md W, Ofuase O. Microorganisms responsible for the spoilage of tomato fruits, *Lycopersicon esculentum*, sold in markets in Benin City, southern Nigeria. *Sch Acad J Biosci* [Internet]. 2014 [cited 2017 Sep 23];2(7):2321-6883. Available from: [www.saspublisher.com](http://www.saspublisher.com)
38. Bello OB, Bello IS, Aminu D, Olawuyi OJ, Afolabi-Balogun NB, Lawal AO, et al. Antibiotic sensitivity of bacterial and fungal isolates from tomato (*Solanum lycopersicum* L.) fruit. *ISSN* [Internet]. 2016 [cited 2017 Sep 22];3(1):2349-9265. Available from: [www.tropicalplantresearch.com](http://www.tropicalplantresearch.com)
39. Adejuwon A. *Alpha Amylases from Fungi Species of Aspergilli and Penicilli*. LAP LAMBERT Academic Publishing; 2013.
40. Godheja J, Shekhar SK, Modi DR. Advances in Molecular Biology Approaches to Guage Microbial Communities and Bioremediation at Contaminated Sites. 2014;2(4):167-77.
41. Sethi S, Datta A, Gupta BL, Gupta S. Optimization of Cellulase Production from Bacteria Isolated from Soil. *ISRN Biotechnol* [Internet]. 2013 Feb 19 [cited 2017 Sep 23];2013:1-7. Available from: [http://www.hindawi.com/journals/isrn/2013/2013/1/1969.PmBG\\_b0d7ac](http://www.hindawi.com/journals/isrn/2013/2013/1/1969.PmBG_b0d7ac)

- 13/985685/
42. Heerd D, Tari C, Fernández-Lahore M. Microbial strain improvement for enhanced polygalacturonase production by *Aspergillus sojae*. *Appl Microbiol Biotechnol* [Internet]. 2014 Sep 4 [cited 2017 Sep 23];98(17):7471–81. Available from: <http://link.springer.com/10.1007/s00253-014-5657-z>
43. Atolagbe OM, Ajayi AA, Edegbo O. Characterization of  $\alpha$ -Amylase from Soursop (*Annona muricata* Linn.) Fruits Degraded by *Rhizopus stolonifer*. *Pakistan J Biol Sci* [Internet]. 2016 Feb 1 [cited 2017 Sep 23];19(2):77–81. Available from: <http://www.scialert.net/abstract/?doi=pjbs.2016.77.81>
44. Fernández Núñez EG, Barchi AC, Ito S, Escaramboni B, Herculano RD, Mayer CRM, et al. Artificial intelligence approach for high level production of amylase using *Rhizopus microsporus* var . *oligosporus* and different agro-industrial wastes. *J Chem Technol Biotechnol* [Internet]. 2017 Mar 1 [cited 2017 Sep 22];92(3):684–92. Available from: <http://doi.wiley.com/10.1002/jctb.5054>
45. Shinde S, Soni R. " Production and Partial Purification of  $\alpha$ -Amylase from Bacterial Strains " *Int J Genet Eng Biotechnol* [Internet]. 2014 [cited 2017 Sep 23];974(1):57–62. Available from: <http://www.irphouse.com>

## REVIEW ARTICLE

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### CANDIDA AURIS INFECTION: HOW PREPARED IS NIGERIA FOR THIS EMERGING FUNGAL AGENT?

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#### ABSTRACT

Reports from Asia and other parts of the world have demonstrated that the incidence of *Candida auris* infection is on the rise. *Candida auris* is a fungal pathogen causing a wide variety of infections affecting people of all age groups. *Candida auris* was first described in 2009 and has since emerged as an important cause of invasive fungal infection, most importantly healthcare-associated candidaemia. Large outbreaks have been reported worldwide, with therapeutic failure and associated high mortality rates recorded.

The emergence and spread of *C. auris* raises public health concerns because of some characteristics of this pathogen. First it is resistant to many antifungal agents making this infection very difficult to treat. It is also associated with horizontal transmission in health care settings causing outbreaks among hospitalized individuals with high mortality rates. Furthermore, identification of *C. auris* is a challenge. Routine identification methods usually misidentify the organism as other yeasts especially *Candida haemulonii*.

This review discusses the current knowledge on the epidemiology, treatment and control of this infection. The urgent need for the stakeholders in Nigerian tertiary hospitals to set machinery in motion for prompt diagnosis, management and prevention of transmission of this infection are also discussed.

### INFECTION À CANDIDA AURIS: COMMENT LE NIGERIA EST-IL PRÉPARÉ POUR CET AGENT FONGIQUE ÉMERGENT?

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#### ABSTRAIT

Des rapports en provenance d'Asie et d'autres parties du monde ont démontré que l'incidence de l'infection à *Candida auris* est en augmentation. *Candida auris* est un pathogène fongique causant une grande variété d'infections affectant les personnes de tous les groupes d'âge. *Candida auris* a été décrit pour la première fois en 2009 et est depuis apparu comme une cause importante d'infection fongique invasive, surtout la candidémie associée aux soins de santé. De grandes épidémies ont été signalées dans le monde entier, avec des échecs thérapeutiques et des taux de mortalité élevés associés enregistrés.

L'émergence et la propagation de *C. auris* soulèvent des préoccupations en matière de santé publique en raison de certaines caractéristiques de ce pathogène. D'abord, il est résistant à de nombreux agents antifongiques rendant cette infection très difficile à traiter. Il est également associé à la transmission horizontale dans les milieux de soins de santé, ce qui provoque des éclosions parmi les personnes hospitalisées avec des taux de mortalité élevés. De plus, l'identification de *C. auris* est un défi. Les méthodes d'identification de routine confondent généralement l'organisme avec d'autres levures, en particulier *Candida haemulonii*.

Cette revue discute les connaissances actuelles sur l'épidémiologie, le traitement et le contrôle de cette infection. Le besoin urgent pour les parties prenantes des hôpitaux tertiaires du Nigeria de mettre en place un système de diagnostic rapide, de prise en charge et de prévention de la transmission de cette infection est également discuté.

#### INTRODUCTION

Candidaemia and candidiasis are usually caused by *Candida albicans* but infections due to non albicans *Candida* species have been on the increase globally. (1) Cases of invasive non albicans candidiasis have been reported from many parts of the world. Among the

non albicans *Candida* species; *C. glabrata* and *C. tropicalis* have emerged as important opportunistic pathogens with other species being reported to a lesser degree.

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In 2009, a novel yeast species belonging to the genus *Candida* was isolated from the external ear canal of a patient admitted in a Japanese hospital. (2) DNA analysis revealed this new species to be closely related *Candida ruelliae* and *Candida haemulonii* in the Metschnikowiaceae clade. It was thereafter named as *C. auris* based on its first isolation from an ear infection. (2)

*Candida auris* is an unusual *Candida* species first found in human ear specimens. It is capable of hospital acquired transmission leading to large outbreak in health care settings. (3) It has emerged as a significant pathogen in hospitals and accounts for 8.6% to 30% of cases of candidaemia in a recent report. (4)

Contaminated surfaces was thought to be the source of dissemination of *C. auris*, however, urogenital colonization from an indwelling urinary catheter could also result in dissemination to the blood stream causing fungemia. (5, 6)

## EPIDEMIOLOGY

*Candida auris* has become an important nosocomial pathogen with widespread dissemination across several Asian countries and other parts of the world. (7) The actual global picture remains unclear as the current commercial methods of laboratory diagnosis misidentify *C. auris* (8)

Since it was first reported in Japan in 2009, *C. auris* has been reported in many other regions of the world. (2) In South Korea, it was isolated in fifteen cases of chronic otitis media and subsequently in three patients with blood stream infections across three different hospitals. (9) Candidaemia caused by *C. auris* has also been reported in India and South Africa, with an estimated prevalence of 0.3%. (10) (6) The first cases in the United Kingdom were recorded in 2013 from blood cultures, thereafter there have been many others documented including outbreaks in 2013 and 2015-2016. (3, 7) In the United States, *C. auris* has been identified from over 122 patients, 77 clinical cases and 45 patient contacts. Majority of the cases were in New York. (11) Whole-genome sequencing of *C. auris* isolates has shown, clustering into four distinct clades. (11) Studies have found that isolates from within each geographical region are highly related to one another whereas isolates from different regions did not exhibit such relatedness. (11, 12) These suggest independent emergence of *C. auris* within geographical regions followed by local transmission. (11, 12) This geographically specific clustering of *C. auris* has also been demonstrated in a study involving samples from the India, South Africa, Japan, Korea and Brazil. (13)

## PATHOGENESIS

*C. auris* has an innate pliability for survival and persistence in the hospital environment; it is able to rapidly colonize patients' skin and is highly transmissible within the healthcare setting. (3). This has led to serious and protracted outbreaks. *C. auris* does not produce hyphae and produces only rudimentary pseudohyphae, however many strains are highly pathogenic with some as pathogenic as *C. albicans*. (7), (14) Various virulence factors are responsible for the ability of *C. auris* to easily survive and persist in infection sites. Phospholipases are extracellular hydrolytic enzymes which help in adherence and invasion of host cells and this has been demonstrated in *C. auris*. (14, 15) *C. auris* also produces hemolysin leading to invasive disease and widespread infection. (15) Proteinase activity has also been demonstrated in *C. auris* and is said to be in higher proportion than phospholipase production. (15) (14)

Some isolates of *C. auris* have been noted to form large aggregates as a result of failure of budding yeast to separate. This observation was found in cases of lethal infection and it is thought that aggregation might be a mode of immune evasion and tissue persistence. (7) (16)

Earlier studies had also reported the lack of biofilm production by *C. auris* compared to its counterpart *C. haemulonii*. (17) Subsequent researches have however demonstrated that *C. auris* is able to differentially adhere to polymeric surfaces, form biofilms, and resist antifungal agents. (18) These biofilms are, however, much thinner than those formed by *C. albicans* and have a limited amount of extracellular matrix. (14) *C. auris* is also able to grow at high temperatures of 37°C–42°C and exhibit lethality and tissue invasion close to that of *C. albicans*, the most pathogenic *Candida* species. (16)

## RISK FACTORS

Individuals at extreme ages have been noted to be at high risk of infection. (9) Presence of foreign bodies such as central venous catheters, urinary catheters and mechanical ventilation also increase the risk of infection. (9) (19) (6) Concomitant use of broad-spectrum antibiotics and use of antifungals puts the patient at higher risk. (6, 13) A case series involving many countries has found intensive care stay to be a major risk factor for *C. auris* infections which is similar to other studies. (3, 13, 19) Comorbidities and immunosuppressive conditions such as diabetes mellitus, chronic kidney disease, cancer chemotherapy, hematologic malignancies, and bone marrow transplantation have also been identified. (1) (20) Additional risk factors are erythrocyte transfusion, parenteral nutrition,

abdominal surgery, hemodialysis, pancreatitis and HIV infection. (9) (13) (19) (20)

Neutropenia does not appear to carry appreciable risk according to available reports. (9), (1)

A study found longer ICU stay, underlying respiratory disease, vascular surgery, medical intervention and exposure to antifungals as the major risk factors for acquiring *C. auris* infection. (21)

### CLINICAL CONDITIONS

*C. auris* is an emerging fungal pathogen that can cause a wide range of human infections especially in intensive care settings (22), (4) The clinical spectrum of *C. auris* infections has expanded from minor cases of superficial infections to highly invasive bloodstream infections. (8) It is a source of multi-resistant health-care associated infections with a high potential for horizontal transmission in the hospital setting. (23) Many studies including that by Schelenz et al have demonstrated such transmission in hospitalized patients. (3) *Candida auris* is a recognized cause of chronic otitis media, wound infections, including diabetic foot. (22) (7). The occurrence of candidaemia due to *C. auris* appears to be on the increase and is associated with high mortality of up to 50%. (3) (7) (11) *C. auris* accounted for 30% of the annual candidaemia cases in a tertiary care general hospital; most of the patients had persistent candidaemia with overall mortality rates of 30- 50%. (6) *C. auris* had also been reported as a cause of bronchopneumonia. (22), (11) Other sites where *C. auris* has been cultured are urine, bile fluid, bone and jejunum. (11)

### LABORATORY DIAGNOSIS

Specimens for laboratory diagnosis depend on clinical presentation and include blood, sputum, swabs, urine and others. On Sabouraud dextrose agar (SDA), colonies of *C. auris* are white to cream colored and smooth. (9), (4) Microscopic examination shows ovoid to globose budding yeast cells in singles or pairs. (4) *C. auris* does not form chlamydoconidia or pseudohyphae on cornmeal agar. (4) (14) It failed to form Chlamydoconidia even after 3 days of growth on Cornmeal agar at 30°C. (6) It grows well at 37 and up to 42°C but does not grow at 45°C. (9) (6) No growth has been observed on cycloheximide-containing medium. (9) Urease test and nitrate assimilation test are both negative in *C. auris*. (9) In contrast to *C. auris*, *C. haemulonii* and *C. duobushaemulonii* isolates produce pseudohyphae and do not grow at 42°C. (4) Assimilation of N-acetylglucosamine (NAG) is quite variable and therefore not valid in differentiating *C. auris* from *C. pseudohaemulonii*. (4) (6) It appears pink on CHROM-agar *Candida* medium and grows at 37°C and 42°C. (6)

Although *C. auris* is close phylogenetically to *Candida haemulonii* and *Candida ruelliae* in the Metschnikowiaceae clade analyses of the 26S rDNA D1/D2 domain, nuclear ribosomal DNA ITS region sequences, and also chemotaxonomic studies have indicated that it represents a new species. The taxonomic description of *Candida auris* sp. nov. was proposed in 2009. (2)

Unfortunately, the usual phenotypic tests are unable to correctly identify *C. auris* which is misidentified as one of the closely related *Candida* species especially *C. haemulonii*.

The Vitek 2 system is unable to differentiate between isolates of *C. auris* and those of *C. haemulonii* and *Candida pseudohaemulonii* identifying them as *C. haemulonii* or *C. famata*. (9), (6), (10)

In addition, the API 20C system, identified isolates of *C. auris* as *Rhodotorula glutinis*, or *C. sake* while *C. haemulonii* and *C. pseudohaemulonii* were identified as *Kodamaea ohmeri*. (9) (6)

Currently, the reliable methods for definitive identification of *C. auris* are molecular based methods such as PCR, sequencing analysis, amplified fragment length polymorphism (AFLP) fingerprinting and MALDI-TOF biotyping. (1, 3, 13) Internal transcribed spacer (ITS) region sequencing is used to confirm the identity of suspected isolates as *C. auris*. (6), (1), (3), (4)

Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can be used to identify related species of *Candida*. (4) AFLP has been shown to reliably distinguish *C. auris* from other closely related species such as *C. haemulonii*, *C. pseudohaemulonii* and *C. duobushaemulonii*. (3) In addition, REAG-N can be used for epidemiologic typing of *C. pseudohaemulonii* and *C. auris* isolates. (17) A cheap method was devised by Kumar et al for identifying *C. auris* among isolates identified by VITEK2 as members of *C. haemulonii* complex. CHROMagar *Candida* medium is supplemented with Pal's agar and on this medium *C. auris* strains show confluent growth of white to cream colored smooth colonies both at 37°C and 42°C without pseudohyphae production. On the contrary, *C. haemulonii* complex isolates show poor growth of smooth, light-pink colonies later becoming semi-confluent with pseudohyphae production. (24)

### ANTIFUNGAL RESISTANCE

*C. auris* has a phylogenetic relationship with *Candida krusei*, *C. haemulonii*, and *C. lusitanae*. These species are known to have either intrinsic or inducible resistance to antifungal agents such as fluconazole and amphotericin B. (12) There are at present no epidemiological cutoff values (ECVs) or clinical

breakpoints defined for *C. auris*, thus non species-specific breakpoints are usually used. (25)

*C. auris* isolates are resistant to fluconazole with high MICs observed in virtually all reports. (6), (1) (10) (4) Among the other azoles, isavuconazole, posaconazole, itraconazole and voriconazole, potent activity against *C. auris* has been demonstrated. (1),(26) The results are however inconsistent as variable results have been observed depending on the study. One study found posaconazole to have the most potent activity in vitro followed by isavuconazole then itraconazole. (25) Reduced susceptibility voriconazole has been demonstrated in some isolates but with excellent activity of isavuconazole and posaconazole. (10), (4) In contrast, another study observed resistance to itraconazole but susceptibility to voriconazole. (15) Flucytosine also shows excellent in vitro activity. (6)

In most investigations, Amphotericin B showed excellent activity against *C. auris* although a few cases of resistance have been observed. (1), (6), (10), (15), (25)

The echinocandins, caspofungin, anidulafungin and micafungin, have excellent activity against the organism.(1, 6, 10) *C. auris* resistant to all classes of antifungals including echinocandin has been reported, thereby emphasizing the importance of antifungal susceptibility testing. (13)

In summary, *C. auris* exhibits uniform resistance to fluconazole, variable susceptibility to the other azole antifungals and a low acquired resistance to amphotericin B and the echinocandins. (25)

#### **ANTIFUNGAL THERAPY**

The first-line therapy is an echinocandin, pending the results of susceptibility testing, which should be carried out without delay. (27) Duration of antifungal therapy is like other infections caused by other *Candida* spp. In candidaemia, treatment should be continued for 14 days after resolution of symptoms attributable to candidaemia and also documented evidence of clearance of *Candida* from the bloodstream. (27) A novel drug, SCY-078, the first orally bioavailable 1, 3- $\beta$ -D-glucan synthesis inhibitor, has demonstrated potent antifungal activity against various *Candida* spp including *C. auris*. (14)

#### **INFECTION PREVENTION AND CONTROL**

Similar to other *Candida* infections, *C. auris* infections appear to be hospital acquired, occurring several days to weeks into a patient's hospital stay. (12, 27) This suggests an exogenous rather than endogenous source of infection and a breach of infection control procedures. In cases of infections, environmental sampling has showed persistent presence of *C. auris* around bed space areas. (3) Implementation of strict

infection control is therefore of paramount importance in the control of this infection. (11)

Cases and their contacts should be cohorted / isolated; and patient housed in a private room. Before de-isolating the patient, a series of three negative screens taken 24 hours apart is advocated. (27) To decrease the risk for transmission, health care personnel in acute care settings should use Standard and Contact Precautions. Personal protective clothing including cuffed long-sleeved disposable gowns, gloves and aprons should be worn by health care workers. (3) Decolonization of *C. auris* infected patients can be performed using chlorhexidine formulations depending on the site. Oral nystatin can also be prescribed if oropharyngeal colonization is present. (3) Environmental decontamination can be implemented using chlorine-based products and hydrogen peroxide vapour. (3)

When transferring colonized patients to other health care facilities, the receiving facilities need to be notified of the presence of this multidrug-resistant organism so as to ensure that appropriate precautions are adhered to. There should be thorough daily and, also on discharge of patient, terminal cleaning of rooms of patients infected with *C. auris* infections, using a disinfectant active against *Clostridium difficile* spores. (11, 20) Equipment used for patients should be cleaned and disinfected with hydrogen peroxide vapour. (3) Screening for *C. auris* in units having patients with ongoing infections or patients coming from other affected hospitals/units or patients at risk for candidiasis may be conducted. Suggested screening sites are: Nose, throat, and groin, urine/urethral swab, perineal or low vaginal swab, sputum/endotracheal secretions, wounds and other appropriate sites. All screen-positive patients should be isolated or cohorted. (27)

#### **PROGNOSIS**

*Candida auris* is an emerging healthcare-associated fungal pathogen associated with high mortality rate of 40-70%. (12) (1) Patients may have therapeutic failure with persistent candidaemia in spite of antifungal treatment which results in fatal outcomes. (6, 9, 20)

Recurrent *C. auris* candidaemia occurring 3 to 4 months after the initial episode of infection is another complication that may be encountered *C. auris*. (20)

Overall outcome is usually better when infection prevention measures are in place. (18)

#### **LEVEL OF PREPAREDNESS IN NIGERIA AND FUTURE DIRECTIVE**

Nosocomial outbreaks as well as recovery of laboratory confirmed isolates of *C. auris* have not been reported in Nigeria. More assertive infection control measures and steps should be put in place to prevent

the transmission of the organism in our health care settings. *Candida auris* infection should be suspected when the isolate is recovered from the critically ill patients in different Intensive Care units (ICU) in our tertiary hospitals. According to the recommendation of Centre for Disease control (CDC), all *Candida* isolates recovered from sterile sites should be identified to the species level so that initial empirical treatment can be administered on species -specific antifungal susceptibility patterns(28, 29). All healthcare facilities in Nigeria with high clinical suspicion of inpatients with *C. auris* should contact the local authority as well as the Federal Ministry of Health (FMoH) for notification.

Medical laboratories should emphasise the importance of accurate species identification for *Candida* to a species level. Many Medical Microbiology laboratories do not routinely speciate non-*Candida albicans* isolates or utilize yeast identification methods such as chromogenic agar, biochemical tests (API) or automated systems such as VITEK which do not speciate this pathogen or may misidentify *C. auris* as yeasts such as *Candida haemulonii*, *Candida sake*, and *Rhodotorula mucilaginosa*.(30) Clinical laboratories should be encouraged to forward *C. haemulonii* isolates and isolates not identified beyond *Candida* spp. by conventional methods to tertiary or public health laboratories in Nigeria for further characterization.

Antifungal resistance is an important concern in managing invasive *Candida* infections, Therefore, understanding drug susceptibility of these different species can help develop protocols for appropriate empirical treatment of these infections. We recommend that each hospital should put in place good antimicrobial stewardship programme and monitor the antifungal susceptibility pattern against this organism. In addition, each hospital should develop their guidelines and policies for the control and prevention of *C. auris* infection.

## CONCLUSION

There should be high level of clinical suspicions when non-*albicans Candida* are isolated in high risk patients admitted in intensive care units. Antifungal susceptibility testing should be initiated and performed routinely in all our tertiary hospitals and public medical microbiology laboratories. If there should be any report of positive cases, infected patients should be cared for in an isolated single room to prevent transmission of the pathogen.

Other aggressive infection control measures should be implemented in the hospitals. These include stringent hand hygiene procedures before and after touching the patients and items around the bed sides; cleaning of affected clinical areas with high strength chlorine-based agents and prompt treatment of patients with antifungal agents.

## REFERENCES

1. Sarma S, Kumar N, Sharma S, Govil D, Ali T, Mehta Y, et al. Candidemia caused by amphotericin B and fluconazole resistant *Candida auris*. *Indian journal of medical microbiology*. 2013;31(1):90-1.
2. Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiology and immunology*. 2009;53(1):41-4.
3. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, et al. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrob Resist Infect Control*. 2016;5:35.
4. Kathuria S, Singh PK, Sharma C, Prakash A, Masih A, Kumar A, et al. Multidrug-Resistant *Candida auris* Misidentified as *Candida haemulonii*: Characterization by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry and DNA Sequencing and Its Antifungal Susceptibility Profile Variability by Vitek 2, CLSI Broth Microdilution, and Etest Method. *J Clin Microbiol*. 2015;53(6):1823-30.
5. Cadnum JL, Shaikh AA, Piedrahita CT, Sankar T, Jencson AL, Larkin EL, et al. Effectiveness of Disinfectants Against *Candida auris* and Other *Candida* Species. *Infect Control Hosp Epidemiol*. 2017;38(10):1240-3.
6. Chowdhary A, Sharma C, Duggal S, Agarwal K, Prakash A, Singh PK, et al. New clonal strain of *Candida auris*, Delhi, India. *Emerging infectious diseases*. 2013;19(10):1670-3.
7. Borman AM, Szekely A, Johnson EM. Comparative Pathogenicity of United Kingdom Isolates of the Emerging Pathogen *Candida auris* and Other Key Pathogenic *Candida* Species. *mSphere*. 2016;1(4).
8. Chatterjee S, Alampalli SV, Nageshan RK, Chettiar ST, Joshi S, Tatu US. Draft genome of a commonly misdiagnosed multidrug resistant pathogen *Candida auris*. *BMC Genomics*. 2015;16:686.
9. Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, et al. First three reported cases of nosocomial fungemia caused by *Candida auris*. *Journal of clinical microbiology*. 2011;49(9):3139-42.
10. Magobo RE, Corcoran C, Seetharam S, Govender NP. *Candida auris*-associated candidemia, South Africa. *Emerging infectious diseases*. 2014;20(7):1250-1.

11. Tsay S, Welsh RM, Adams EH, Chow NA, Gade L, Berkow EL, et al. Notes from the Field: Ongoing Transmission of *Candida auris* in Health Care Facilities - United States, June 2016-May 2017. *MMWR Morb Mortal Wkly Rep.* 2017;66(19):514-5.
12. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous Emergence of Multidrug-Resistant *Candida auris* on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses. *Clin Infect Dis.* 2017;64(2):134-40.
13. Sharma C, Kumar N, Pandey R, Meis JF, Chowdhary A. Whole genome sequencing of emerging multidrug resistant *Candida auris* isolates in India demonstrates low genetic variation. *New Microbes New Infect.* 2016;13:77-82.
14. Larkin E, Hager C, Chandra J, Mukherjee PK, Retuerto M, Salem I, et al. The Emerging Pathogen *Candida auris*: Growth Phenotype, Virulence Factors, Activity of Antifungals, and Effect of SCY-078, a Novel Glucan Synthesis Inhibitor, on Growth Morphology and Biofilm Formation. 2017;61(5).
15. Kumar D, Banerjee T, Pratap CB, Tilak R. Itraconazole-resistant *Candida auris* with phospholipase, proteinase and hemolysin activity from a case of vulvovaginitis. *J Infect Dev Ctries.* 2015;9(4):435-7.
16. Ben-Ami R, Berman J, Novikov A, Bash E, Shachor-Meyouhas Y, Zakin S, et al. Multidrug-Resistant *Candida haemulonii* and *C. auris*, Tel Aviv, Israel. *Emerg Infect Dis.* 2017;23(1).
17. Oh BJ, Shin JH, Kim MN, Sung H, Lee K, Joo MY, et al. Biofilm formation and genotyping of *Candida haemulonii*, *Candida pseudohaemulonii*, and a proposed new species (*Candida auris*) isolates from Korea. *Med Mycol.* 2011;49(1):98-102.
18. Sherry L, Ramage G, Kean R, Borman A, Johnson EM, Richardson MD, et al. Biofilm-Forming Capability of Highly Virulent, Multidrug-Resistant *Candida auris*. *Emerg Infect Dis.* 2017;23(2):328-31.
19. Morales-Lopez SE, Parra-Giraldo CM, Ceballos-Garzon A, Martinez HP, Rodriguez GJ, Alvarez-Moreno CA, et al. Invasive Infections with Multidrug-Resistant Yeast *Candida auris*, Colombia. *Emerging infectious diseases.* 2017;23(1):162-4.
20. Vallabhaneni S, Kallen A, Tsay S, Chow N, Welsh R, Kerins J, et al. Investigation of the First Seven Reported Cases of *Candida auris*, a Globally Emerging Invasive, Multidrug-Resistant Fungus - United States, May 2013-August 2016. *MMWR Morb Mortal Wkly Rep.* 2016;65(44):1234-7.
21. Rudramurthy SM, Chakrabarti A, Paul RA, Sood P, Kaur H, Capoor MR, et al. *Candida auris* candidaemia in Indian ICUs: analysis of risk factors. *The Journal of antimicrobial chemotherapy.* 2017;72(6):1794-801.
22. Chowdhary A, Anil Kumar V, Sharma C, Prakash A, Agarwal K, Babu R, et al. Multidrug-resistant endemic clonal strain of *Candida auris* in India. *Eur J Clin Microbiol Infect Dis.* 2014;33(6):919-26.
23. Calvo B, Melo AS, Perozo-Mena A, Hernandez M, Francisco EC, Hagen F, et al. First report of *Candida auris* in America: Clinical and microbiological aspects of 18 episodes of candidemia. *J Infect.* 2016;73(4):369-74.
24. Kumar A, Sachu A, Mohan K, Vinod V, Dinesh K, Karim S. Simple low cost differentiation of *Candida auris* from *Candida haemulonii* complex using CHROMagar *Candida* medium supplemented with Pal's medium. *Rev Iberoam Micol.* 2017;34(2):109-11.
25. Arendrup MC, Prakash A, Meletiadis J, Sharma C, Chowdhary A. Comparison of EUCAST and CLSI Reference Microdilution MICs of Eight Antifungal Compounds for *Candida auris* and Associated Tentative Epidemiological Cutoff Values. *Antimicrob Agents Chemother.* 2017;61(6).
26. Chowdhary A, Sharma C, Meis JF. *Candida auris*: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLoS Pathog.* 2017;13(5):e1006290.
27. Sarma S, Upadhyay S. Current perspective on emergence, diagnosis and drug resistance in *Candida auris*. *Infect Drug Resist.* 2017;10:155-65.
28. Chowdhary A, Voss A, Meis J. Multidrug-resistant *Candida auris*: 'new kid on the block' in hospital-associated infections? *Journal of Hospital Infection.* 2016;94(3):209-12.
29. Control CfD, Prevention. Clinical alert to US healthcare facilities—June 2016. Global emergence of invasive infections caused by the multidrug-resistant yeast *Candida auris*. 2016 [cited 2016 Jun 24].
30. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A, Chowdhary A, Hall A, et al. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. *Antimicrobial Resistance & Infection Control.* 2016;5(1):35.

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### RESULT OF 2004 EXTENDED TRYPANOSOMOSIS SURVEY OF RUMINANTS AT KACHIA GRAZING RESERVE, NORTH CENTRAL NIGERIA

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#### Running Title: Ruminant Trypanosomosis Prevalence in Nigeria

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#### ABSTRACT

Tsetse fly and trypanosome prevalence in ruminants were estimated in April and August, peak months of the dry and rainy seasons in the Kachia Grazing Reserve (KGR) of Kaduna State, North Central Nigeria. This study was subsequent to reports of seasonal outmigration of semi nomadic Fulani from the grazing reserve due to death of cattle from trypanosomosis. Result of blood samples showed an overall parasitological infection rate of 17.4%. Infection rates in cattle, sheep and goats were, 18.6%, 9.5% and 5.1% respectively. Over all higher infection rate in the rainy season was attributed to abundance of tsetse and other hematophagous flies. Infection rate in younger animals (21.9%) was higher compared to those of older animals (16.5%). *Trypanosoma vivax* was the dominant infecting trypanosome species followed by *T. congolense* and *T. brucei*.

It was concluded that tsetse fly and trypanosomosis constituted dual plagues limiting economic livestock production and settling of the pastoralists in the grazing reserve. This warrants application of sustainable integrated control measures to enhance utilization of abundant fodder at the reserve.

Key words: Kachia grazing reserve, trypanosomosis, ruminants, infection rates, Nigeria.

### RÉSULTAT DE L'ENQUÊTE DE TRYPANOSOMOSE EXTENSION DE 2004 DES RUMINANTS À LA RÉSERVE DE PISCINE KACHIA, NIGERIA CENTRALE DU NORD

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#### ABSTRAIT

La prévalence de la mouche tsé-tsé et du trypanosome chez les ruminants a été estimée en avril et août, les mois de pointe des saisons sèches et pluvieuses dans la réserve de pâturage de Kachia (KGR) de l'État de Kaduna, dans le nord du centre du Nigeria. Cette étude a été postérieure à des rapports d'émigration saisonnière de Fulani semi-nomades provenant de la réserve de pâturage en raison de la mort de bovins de la trypanosomose. Le résultat des échantillons de sang a montré un taux global d'infection parasitaire de 17,4%. Les taux d'infection chez les bovins, les ovins et les chèvres étaient respectivement de 18,6%, 9,5% et 5,1%. Le taux d'infection plus élevé pendant la saison des pluies a été attribué à l'abondance de mouches tsé-tsé et d'autres mouches hématophobes. Le taux d'infection chez les animaux plus jeunes (21,9%) était plus élevé par rapport à ceux des animaux plus âgés (16,5%).  
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*Trypanosoma vivax* était le trypanosome infectant dominant suivi de *T. congolense* et *T. brucei*.

On a conclu que la mouche tsé-tsé et la trypanosomose constituaient des fléaux doubles limitant la production d'élevage économique et la colonisation des pasteurs dans la réserve de pâturage. Cela justifie l'application de mesures de contrôle intégrées durables pour améliorer l'utilisation de fourrages abondants dans la réserve.

Mots clés: réserve de pâturage de Kachia, trypanosomose, ruminants, taux d'infection, Nigeria.

## INTRODUCTION

Tsetse flies and Animal African trypanosomosis constitute major threats to livestock value chains and food security with resultant underdevelopment in several parts of sub-Saharan Africa including Nigeria, in spite of decades of attempts at chemotherapeutic and vector control (1, 2). In Nigeria, tsetse flies had been shown to infest about 80% of the nation's agro ecological land mass including the high lands of Jos, Mambilla and Obudu plateau, previously known to be tsetse free (3). Impact of the disease arise from not less than 3 million livestock deaths in Africa each year and reduced calving rate, livestock numbers, milk off-take, meat off take, animal draft power and mixed farming (1, 4). Conflict among farmers and pastoralists induced by freshwater scarcity (5) has strengthened the need for development of Nigeria's grazing reserves as panacea. The Nigerian Institute for Trypanosomiasis Research had over the years received reports of outbreaks of animal trypanosomosis in southern parts of Kaduna State (6) with resultant deaths of cattle and migration of semi nomadic Fulani out of the area. For this reason an extended survey was undertaken in the Kachia Grazing Reserve, located within this area, to assess the status of trypanosome infection in ruminants in the grazing reserve for application of appropriate control measures that will permit the settling of more herds in this area and exploitation of the abundant fodder for large scale livestock production. This study was undertaken in 2004 but could not be published due to problems initially encountered in the retrieval of data from storage systems. Even though many works have since been done in this reserve, the data is here presented for the purpose of referencing.

## MATERIALS AND METHODS

**2.1 Study Area**  
The Kachia (Ladduga) Grazing Reserve (KGR) is

situated at Latitude 10°03' and 10°13'N and Longitude 7°55' and 8°06'E within the Northern Guinea savanna (Fig. 1) and spans an area of 88,411 hectares, forming parts of Kufana, Kachia and Kamuru Ikulu lands in Chikun, Kachia and Zangon Kataf Local Government Areas respectively in Kaduna State, North Central Nigeria. The Grazing Reserve is divided into six blocks each comprising of an earth dam (Fig. 2) used for the watering of animals. The reserve is inhabited by settled semi nomadic Fulani who apart from cultivation of crops like maize, guinea corn, millet, yams, cocoa yam, groundnuts and cassava, keep cattle, sheep and goats. The Reserve comprised also of nomadic primary and secondary schools, a market, veterinary clinic and health center at the central area of the Reserve. The animals settle here in most part of the year and migrate out to neighboring states following the drying up of the dams and streams at the onset of the dry season which begins in October.

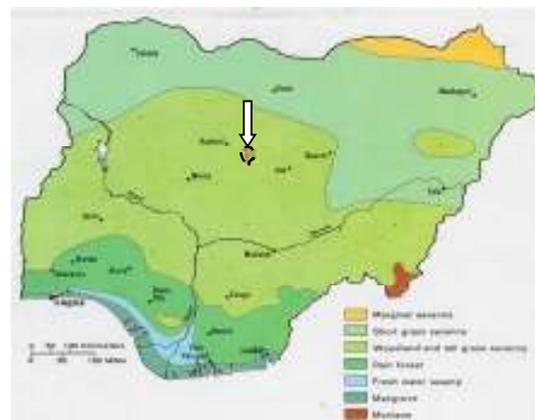


FIG. 1: VEGETATION MAP OF NIGERIA SHOWING THE KACHIA GRAZING RESERVE (ARROWED) IN THE WOODLAND AND TALL GRASS SAVANNAH

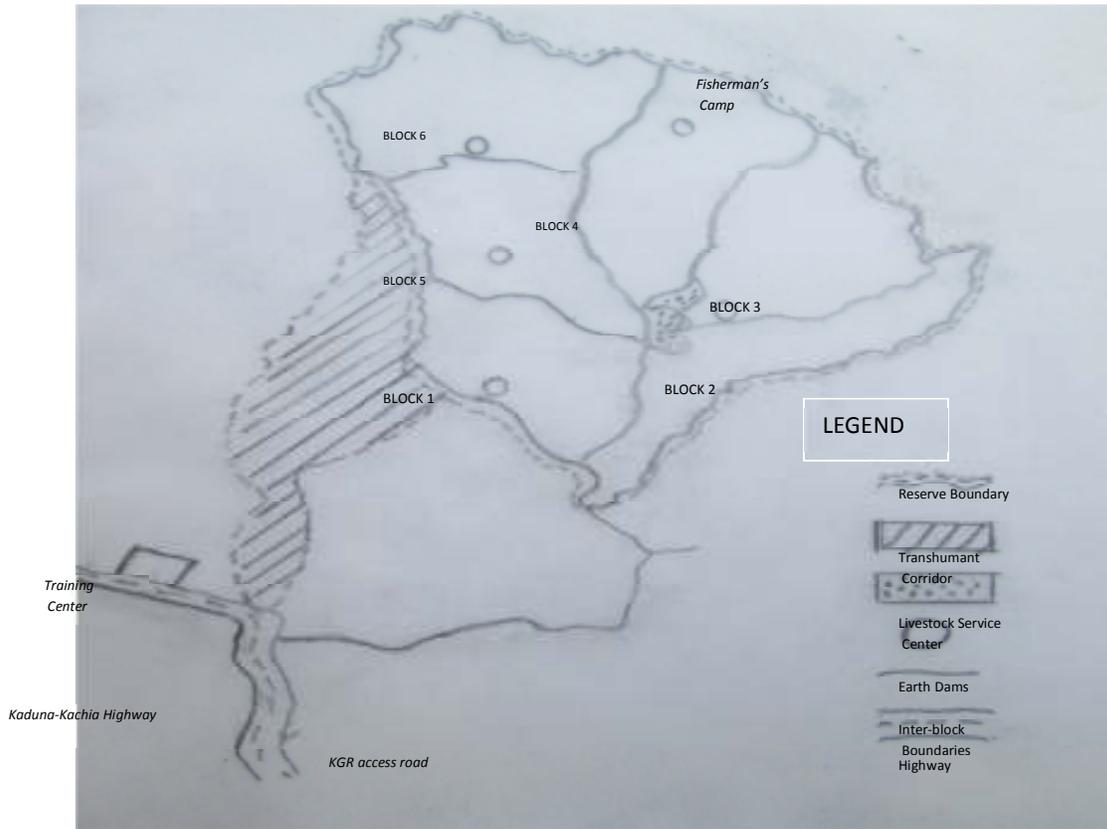


FIG. 2: MAP OF KACHIA GRAZING RESERVE SHOWING GRAZING BLOCKS SAMPLED AND THEIR EARTH DAMS

## Animal Sampling

A systematic random sampling of all animals was used to obtain a sample size of 1,641 animals from all blocks made up of 1445 cattle, 137 sheep and 59 goats. Cattle of one year and below were considered young calves whereas those over one year old were regarded as adults while sheep and goats under 5 months were regarded as young and those above 5 months old as adults. The cattle were made up mostly of White Fulani (Bunaji) breed while those of sheep and goats were those of the Yankasa and Red Sokoto breeds respectively. From each of the animals, 5 milliliters of jugular blood were obtained into specimen bottles containing ethylene diamine tetra acetic acid (EDTA) dispensed as one milligram powder per milliliter of blood and conveyed in cold boxes with ice packs to the field laboratory for analysis. The samples were analyzed using buffy coat dark ground/ phase contrast technique (7) and Giemsa-stained thin smears. Trypanosome species were identified based on their morphological structures from Giemsa-stained thin films. Consent was sought from the Kaduna State Ministry of Agriculture and Natural Resources, Chairman of Kachia Local Government as well as the herd owners and their chiefs before the animals were sampled. All parasitological positive cases were treated the following day with Diaminazene aceturate (Berenil) at the dose of 3.5mg /Kg body weight. The study covered the months of April and August which were peak periods of the dry and wet seasons respectively.

### Statistical analysis

The data analysis was done using Microsoft Excel 2010. Values between infected and uninfected animals were compared using student t - test. Values between the infected ruminant species were compared using one way Analysis of Variance (ANOVA). In all cases values of  $P < 0.05$  were considered significant.

## RESULTS

The trypanosome infection rates in all ruminants sampled from the Kachia Grazing Reserve is summarized on Table I. The overall infection rate in ruminants was 17.4%. Higher infection rate of 18.9% was observed in the rainy season as against 15.7% in the dry season. This was the general pattern in all ruminant species sampled. Infection rate of 18.6% ( $P < 0.05$ ) in cattle was highest followed by 9.5% and 5.1% in sheep and goats respectively. Infection rate in young animals (21.9%, Table II) was higher compared to that of adult animals (16.5%). Infection rate in females (17.8%) was similarly higher than that in males (16.0%, Table III). Of the total number of

parasitological positive infections, 94.3% was due to single infections while 5.8% was due to mixed infection. Infections due to *T. vivax*, *T. congolense*, *T. brucei* were 44.6%, 37.8% and 0.5% respectively. Of the mixed infection cases, 30.8% was due to *T. brucei* and *T. congolense*, 38.5% due to *T. vivax* and *T. brucei*, and 30.8% due to *T. vivax* and *T. congolense* mixed infections. Ruminant population encountered in the rainy season was more than those observed in the dry season as many herds migrated out of the Reserve in search of water as most of the dams and seasonal streams had dried up at the peak of the dry season between the months of March and April.

**TABLE I: TRYPANOSOME INFECTION RATES IN RUMINANT SPECIES AT THE KACHIA GRAZING RESERVE.**

Ruminant Species	Season	No. Animals	No. Positive	Infection Rate(%)
Cattle	Dry	682	116	17.0
	Rainy	763	153	20.1
		(1445)	(269)	18.6
Sheep	Dry	34	2	5.9
	Rainy	103	11	10.7
		(137)	(13)	9.5
Goats	Dry	36	0	0.0
	Rainy	23	3	13.0
		(59)	(3)	5.1
Total	Dry	752	118	15.7
	Rainy	889	167	18.9
		(1641)	(285)	17.4

**TABLE II: AGE DEPENDENT TRYPANOSOME INFECTION RATES AMONG RUMINANT SPECIES AT KACHIA GRAZING RESERVE**

Ruminant Species	Age	No. Animals	No. Positive	Infection Rate (%)
Cattle	Adult	1173	208	17.7
	Young	272	61	22.4
Sheep	Adult	137	13	9.5
	Young	0	0	0.0
Goats	Adult	52	3	5.8
	Young	7	0	0.0
Total	Adult	1362	224	16.5
	Young	279	61	21.9

**TABLE III: SEX DEPENDENT TRYPANOSOME INFECTION RATES AMONG RUMINANT SPECIES AT KACHIA GRAZING RESERVE**

Ruminant Species	Sex	No. Animals	No. Positive	Infection Rate (%)
Cattle	Male	409	68	16.8
	Female	1036	201	19.4
Sheep	Male	38	5	13.2
	Female	99	8	8.1
Goats	Male	17	1	5.9
	Female	42	0	0.0
Total No.	Male	464	74	16.0
	Female	1177	209	17.8

## DISCUSSION

This study showed that tsetse and animal trypanosomosis were prevalent in the Kachia Grazing Reserve. The overall trypanosome infection rate of 17.4% in ruminants was by far higher than the 4.3% over all prevalence rate for Nigeria obtained from the country wide survey by EEC-Trypanosomiasis

control project between 1980- 1996 (3). This suggests that trypanosomosis was a major problem in the grazing reserve. Higher infection rate recorded in cattle than in small ruminants suggests the preference of tsetse and other biting flies for bovine blood than those of small ruminants or the larger size of moving cattle attracted more biting flies compared to small ruminants which are smaller in size. Differences in the infection rates in sheep and goats suggest that husbandry practice played roles in trypanosomosis risk in small ruminants. Whereas sheep were taken along with cattle for grazing, goats were tied around hamlets during the dry and rainy seasons thereby leading to more exposure of the sheep to infection than goats.

Higher infection rates were observed in the rainy season compared to dry season suggesting that this was associated with concurrent increase in tsetse infestation and that of other biting flies at the reserve there by making the risk of trypanosomosis higher in the rainy season. The role of rainy season in abundance of tsetse and other biting flies in trypanosomosis outbreak in livestock in Kaduna State had been described by Maikaje (8). Further analysis of the result showed that trypanosome infection of goats occurred only in the rainy season suggesting that there was encroachment of tsetse on settlement areas which also exposed humans to tsetse bites and trypanosomiasis (sleeping sickness). Kaduna State previously known to be an endemic focus for the human disease was controlled to below endemic proportions (9, 10). Although there had been general resurgence in sleeping sickness in parts of the country (11), the true situation in Kaduna State is not known. Trypanosome infection rate at the grazing reserve was also higher in young animals which suggests that these were current infections acquired probably from the Grazing Reserve with consequent effects on rearing of livestock in the reserve. This may have resulted in reduced herd sizes from death of young animals, growth retardation and infertility in the older animals (1).

The dominance of *T. vivax* infection in the Grazing Reserve may equally have been as a result of prevalent infestation of the area by other biting flies beside tsetse as these have been associated with prevalence of *T. vivax* in the absence of tsetse flies and spread of African trypanosomes beyond tsetse belts of Africa and parts of Asia and Latin America (12, 13).

## CONCLUSION

Trypanosomosis was prevalent in Kachia Grazing Reserve and constituted a major threat to economic

production of livestock in the area with higher infection rates in the rainy season, young and female animals. This called for strategic and integrated seasonal approaches to tsetse and other hemophagous flies as well as trypanosomiasis control in order to limit the impact of the disease and enhance the settling of more pastoralists in the grazing reserve. Integrated seasonal approach to tsetse fly control means the application of different tsetse fly control techniques which include use of insecticide impregnated screens, epicutaneous application of insecticides and Sterile Insect Technique depending on patterns of tsetse fly distribution which varies between dry and wet seasons. This will help limit the

conflicts among farmers and pastoralists in this part of Kaduna state and other parts of the country. At the time of this study there was no sustainable tsetse fly control programme in the grazing reserve and the herdsmen migrated out of the reserve seasonally.

#### CONFLICT OF INTEREST

The authors declare that there was no conflict of interest in the course of this investigation.

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#### REFERENCES

1. Swallow B.M. Impact of trypanosomiasis on African agriculture. PAAT Technical and Scientific Series No. 2; Food and Agriculture Organization of the United Nations, Rome, 2000.
2. Eisler M.C., Dwinger R.H., Majiwa P.A.O., Picozzi K. Diagnosis and epidemiology of African Animal trypanosomiasis. In : The Trypanosomiasis. I. Maudlin, P.H. Holmes and M. A. Miles (eds). CABI Publishing, U.K.PP.253-268, 2004.
3. Onyiah J.A. African animal trypanosomiasis: An overview of the current status in Nigeria. *Trop. Vet.* 1997, 15:111-116.
4. Shaw A.P.M. Economics of African trypanosomiasis. In. The Trypanosomiasis. CABI Publishing, U.K. PP. 369-402, 2004.
5. Adamu U. O., Haruna M. K., Ovbagbedia R. P., Bizi R., Bejanmin W., Malal U. A., Enwezor F. N. C., Muhammed M. Control of African trypanosomiasis in Nigeria: Time to strengthen integrated approaches. *Int. J. Anim. Vet. Adv.* , 2013, 3(3):138 - 143.
6. Abenga J.N., Enwezor F.N.C., Lawani F.A.G., Osue H.O., Ikemareh E.C.D. Trypanosome prevalence in cattle in Lere Area in Kaduna State, North Central Nigeria. *Revue Elev. Med. Vet. Pays Trop.*, 2004, 57:45-48
7. Kalu A. U., Edeghere H. U., Lawani F. A. comparism of diagnostic techniques during sub-clinical single infections of trypanosomiasis in goats. *Vet. Pathol.* , 1986, 22: 27 - 47.
8. Maikaje D.B. An outbreak of biting flies and bovine trypanosomiasis in Kaura LGA, Kaduna State, Nigeria. *West Afr. J. Biol. Sci.* , 2002,13:56-65
9. Duggan A. J. A Survey of Sleeping Sickness control in Northern Nigeria from the earliest time to the present day. *Trans. R. Soc. Trop. Med. Hyg.* ,1962, 55:439-480.
10. Maclean K. J. R. The epizootiology of trypanosomiasis. In African Trypanosomiasis. H.W. Mulligan (ed). George Allen and Unwin/ ODA, London, U.K.PP.751-765, 1970
11. Edeghere H., Olise P.O., Olatunde D.S. Human trypanosomiasis (sleeping sickness): New endemic foci in Bendel State, Nigeria, *Trop. Med, Parasitol.* ,1989, 40: 16-20.
12. Jones T.W., Davila A.M.R. *Trypanosoma vivax* -out of Africa (Review). *Trends Parasitol.* 2001, 17:99-101.
13. Hall M.J.R., Wall R. Biting flies: Their role in the mechanical transmission of trypanosomoses, I. Maudlin, P.H. Holmes and M.A. Miles (eds). CABI Publishing, U.K. PP. 583-694, 2004.

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### FIRST MOLECULAR INVESTIGATION OF CAPSULAR SEROTYPING AND HYPERVIRULENT (HVLP) OF *K. PNEUMONIAE* IN UNIVERSITY HOSPITAL CENTER OF YOPOUGON COTE D'IVOIRE

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#### ABSTRACT

*Klebsiella pneumoniae* is a well known human pathogen. Although infectious in most nosocomial infections with a high level of resistance, capsular types and circulating hypervirulent strains in our context are not documented. The aims of this study are to identify capsular serotypes and hypervirulent strains circulating at the Yopougon University Hospital in Abidjan. 51 strains of *Klebsiella* were collected at Chu de Yopougon. The capsular serotypes were determined using PCR and the serotypes K1, K2 and K5 were searched. The hypervirulent strains were also investigated by PCR and by string test. The predominant serotypes were non-K1 / K2 (46/51, 90%). The serotypes found K5 and K2 in (4/51, 7.8%) and (1/51; 1.9%) respectively. The *rmpA* gene linked to hyperviscosity or hyperviscosity was not found although 25.5% (12/51) were positive for the stretch test. The capsular distribution of strains of *Klebsiella pneumoniae* seems different from Asian authors. The determination of non-K1non types K2 remains to be elucidated.

Keywords: *Klebsiella pneumoniae*, capsular serotype - hypervirulence

### PREMIERE ETUDE D'INVESTIGATION MOLECULAIRE DE SEROTYPAGE CAPSULAIRE ET DE GENE D'HYPERVIRENCE DE *KLEBSIELLA PNEUMONIAE* AU LABORATOIRE DU CHU DE YOPOUGON EN COTE D'IVOIRE

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#### Résumé

*Klebsiella pneumoniae* est un pathogène nosocomial humain bien connu. Bien qu'incriminé dans la plus part des infections nosocomiales avec un niveau élevé de résistance, les types capsulaires et les souches hypervirulentes circulantes dans notre contexte ne sont pas documentés. L'objectif de cette étude est d'identifier les sérotypes capsulaires et les souches hypervirulentes circulant au CHU de Yopougon Abidjan., 51 souches de *Klebsiella* ont été collectés au Chu de Yopougon. Les sérotypes capsulaires ont été déterminée à l'aide de la PCR et les sérotypes K1, K2 et K5 ont été recherchés. Les souches hypervirulentes ont été recherchées également par PCR et par le test d'étirement ou string test. Les sérotypes prédominants étaient les non K1/K2 (46/51; 90%). Les sérotypes retrouvés K5 et K2 dans respectivement (4/51; 7,8%) et (1/51 ; 1,9%). Le gène *rmpA* lié à l'hyperviscosité n'a pas été retrouvé bien que 25,5% (12/51) étaient positives au test d'étirement. La distribution capsulaire des souches de *Klebsiella pneumoniae* semble différente des auteurs asiatiques. D'ou l'intérêt de travaux plus approfondies afin de déterminer les types capsulaire des souches non K1 non K2.

Mots clefs : *Klebsiella pneumoniae* - serotype capsulaire - Hypervirulence

#### INTRODUCTION

*Klebsiella pneumoniae* is an opportunistic pathogen responsible for community infections and nosocomial infections such as pneumonia, septicemia, suppurative and urinary infections, particularly in patients admitted to intensive care (1).

The capsule is considered to be a major virulence factor for *Klebsiella*. It intervenes in the formation of biofilm and in the increase of the anti-opsonised effect allowing the bacterium to escape the immune response of the host (2,3,4)

Currently, 78 capsular antigens of *K. pneumoniae* are listed and the serotypes most frequently involved in human infection are serotypes K1, K2, K5, K54

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Several reports have revealed that capsular types Some of these have reported a correlation between serotypes K1, K2 and abscesses of the liver, but serotypes are not the only determining factor in the occurrence of liver abscesses (4). Although the first case of a patient with liver abscess was described in China in the 1980s (5), this strain of *K pneumoniae* responsible for liver abscess has been reported in Taiwan, Japan, Europe, North America, and Korea. (6,7,8). This characterized emergent infection is often complicated by septic meningitis and purulent endophthalmitis. This new strain called "*Klebsiella pneumoniae hypervirulent* or HvKP" is a variant of the classical strain in terms of aspects of the colonies on the different agar plates. These strains are characterized by a hyperproduction of the capsule mediated by the *rmpA / rmpA2* gene which gives these strains a hyperviscous aspect. The association of hyperviscosity with the presence of the *magA* gene is also found, particularly in strains of serotypes K1, K2, K5, K20, K54 and K57 (9,10) In Ivory Coast, *K pneumoniae* is involved in various infections (11) in human infection and in the colonization of area in hospitals (12). 2010. Although the antibiotic resistance of *Klebsiella pneumoniae* has often been studied (13), data on circulating serotypes and hypervirulent strains are non-existent. The aims of this study are to identify capsular serotypes and hypervirulent strains circulating at the Yopougon University Hospital in Abidjan.

## MATERIALS AND METHODS

are related to the severity of infection. This study was carried out at the Bacteriology-Virology Unit of the Central Laboratory of the Yopougon University Hospital for Classical Bacteriology and at the Molecular Biology Platform of the Pasteur Institute of Côte d'Ivoire for the molecular analysis of the strains of January To March 2016.

### 14. Study population

51 strains of which 47 were of human origin and four of environmental origin (1, 2, 34, and 36) were studied. 7 strains of *K oxytoca*, (17, 24, 33, 44, 48, 53, 55) were associated in molecular analyzes to investigate the specificity of *K pneumoniae* identification primer

### 15. Bactériologie

Isolates were biochemically identified by conventional bacteriology tests. Susceptibility testing for the strains was performed using the Kirby-Bauer disk diffusion test on Mueller-Hinton agar. Results were interpreted according to the recommendations and definitions from Comité Antibiogramme de la Société Française de Microbiologie (CASFM 2016) *E. coli* ATCC 25922 was used as the MIC reference strain.

A string test was performed to distinguish hvKP from cKP according to Fang study [8].

### 16. PCR assay

The "*K.pneumoniae* Pf/*K.pneumoniae* Pr1 "primer pair included in the multiplex PCR is for the identification of *K. Pneumoniae*. The following genes were targeted: *rmpA*, and the K1 K2 and K5 capsular serotypes. The primers targeting the capsular serotype specifying genes were previously described (Table 1).

TABLE 1: PRIMERS USED IN THE MULTIPLEX PCR

SEROTYPE	PRIMER	SEQUENCE	PRODU CT SIZE (PB)	REFERENCES
K1	MAGAF1	GGTGCTCTTACATCATTGC	1283	FANG ET AL. (2004)
	MAGAR1	GCAATGGCCATTGCGTTAG		
K2	K2WZY-F1	GACCCGATATTCATACTTGACAGAG	641	TURTON ET AL. (2008)
	K2WZY-R1	CCTGAAGTAAATCGTAAATAGATGGC		
K5	K5WZXF360	TGGTAGTGATGCTCGCGA	280	TURTON ET AL. (2008)
	K5WZXR639	CCTGAACCCACCCCAATC		
RMPA	RMPAF	ACTGGGCTACCTCTGCTTCA	516	NADASY ET AL. (2007)
	RMPAR	CTTGCATGAGCCATCTTCA		
<i>K.PNEUMONIAE</i> 16S-23S ITS	PF	ATTGAAGAGGTTGCAACGAT	130	LIU ET AL. (2008)
	PR1	TTCACTCTGAAGTTTCTGTGTC		

Extraction of DNA was carried out by thermal shock by a freezing cycle (-20 ° C. for 1 hour and then heating on thermo block for 10 minutes at 95 ° C. The GoTaq G2 Flexi DNA polymerase kit

(Promega Corporation, USA) was used for the PCR mixes containing 0.2µM of each primer, 7.5µM, MgCl, 0.5µM dNTPs, 3 unit Taq polymerase, 1X of buffer and 5µl of DNA template for a final volume

of 50µl. Amplification conditions were :95°C 15 min (1 cycle), (95°C 30 s ,58°C 90 s, 72°C 90s) (35cycles), 72°C 10 min (1 cycle). The revelation was made on a GelDoc Bioanalyzer (BioRad) after electrophoresis on 1.5% agarose gel.

## RESULTS

Concerning the source of ours isolates, (92%) were isolated from clinical sources and (8 %), from hospital environment. 31% were isolated from biological products in the pediatric and 23% from intensive care unit. Of the 47 clinical isolates, 33(65%) were from urine and 7 (14%) from sputum (tableau II)

TABLE II: DISTRIBUTION OF BACTERIAL BY SPECIMEN AND WARDS

Wards	Value (n)	%
Surgery	3	6
Endocrinology	7	14
Médecine	3	6
Nephrology	4	8
Pédiatric	16	31
Intensive care unit	12	23
Over	6	12

Specimen	Value (n)	%
Aspirates	2	4
Catheter tip	2	4
hospital's environment	4	8
CSF	1	2
Sputum	7	14
Blood	2	4
Urine	33	65
Total	51	100

The bacterial isolates exhibited a high resistance to the antibiotics tested. In our study 14% of strains are resistant to at least three families of antibiotic at a time. The proportion of resistance to third generation céphalosporins, ciprofloxacin and gentamicin was 49%, 45% and 33%, respectively, (Table 5). The prevalence of ESBL producing strains was 30% in *K. pneumoniae*

TABLEAU III: DISTRIBUTION OF ANTIBIOTICS RESISTANCE

Antibiotic	Value (n)	%
Amoxicilline+Acide clavulaniqueR	25/51	49
CefoxitineR	15/51	29,5
CeftriaxoneR	25/51	49
ImipénèmeR	1/51	2
CiprofloxacinR	23/51	45
GentamycineR	17/51	33
AmikacineR	2/51	3,9
FosfomycineR	2/51	3,9

The molecular identification all (41) isolates gave positive results and identified as *K. pneumoniae*. Results of PCR amplification confirmed that all isolates were *K. pneumoniae*. Serotype of 9.8% where be identified by primers used whose 7.8% of K2 (Figure 1 and 2) and 2% of K5. Five of identified serotype came from urine 12% (4/33) and sputum 14,28% (1/7) .Of the 5 strains serotyped 2 had a positive String test (40%) however *rpmA* gene linked to hyperviscosity has not been found.

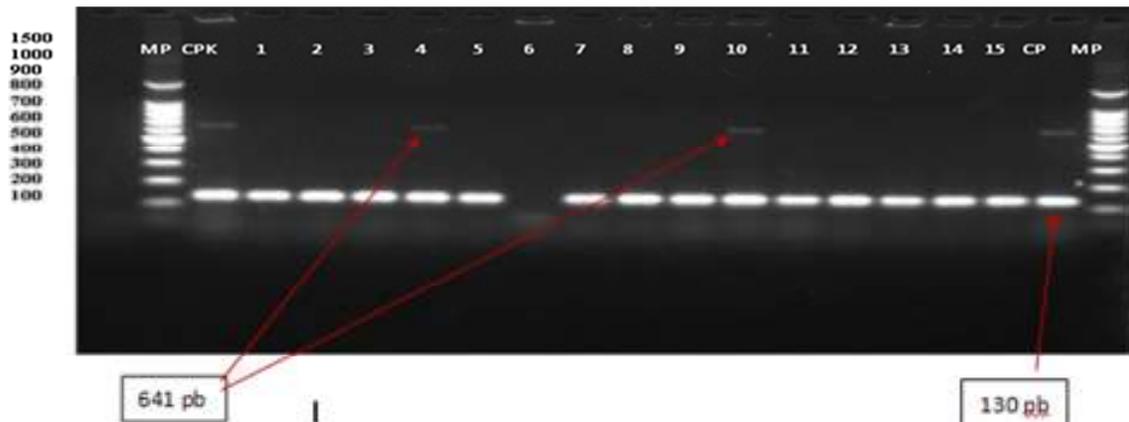


FIGURE 1: STRAINS 1 AND 15 WITH SEROTYPE K2 (PB = 641)

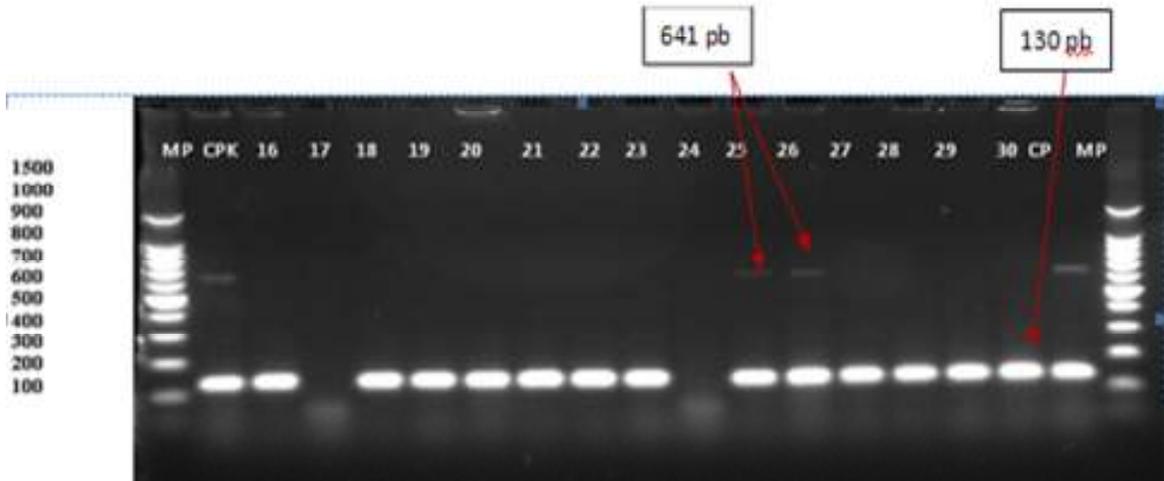


FIGURE 2: STRAINS 16 AND 30 WITH SEROTYPE K2 ( PB = 641)

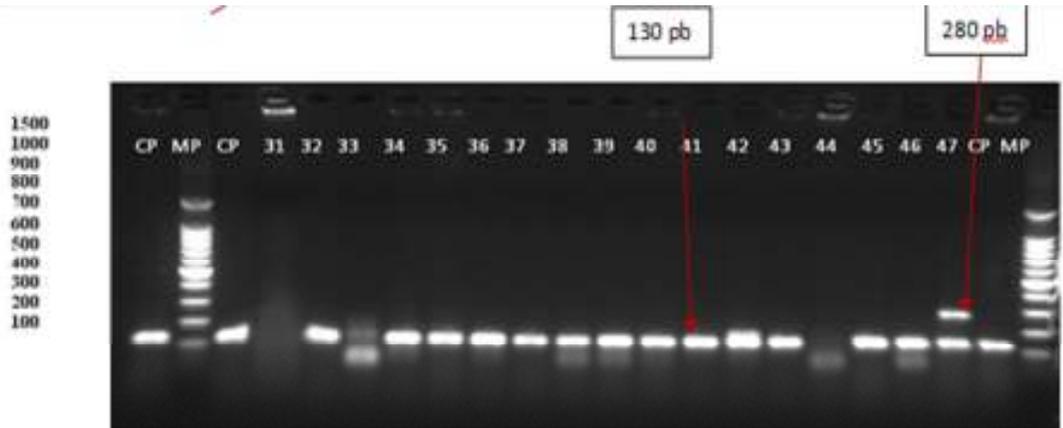


FIGURE 3: STRAIN 47 WITH SEROTYPE K5 (PB = 280)

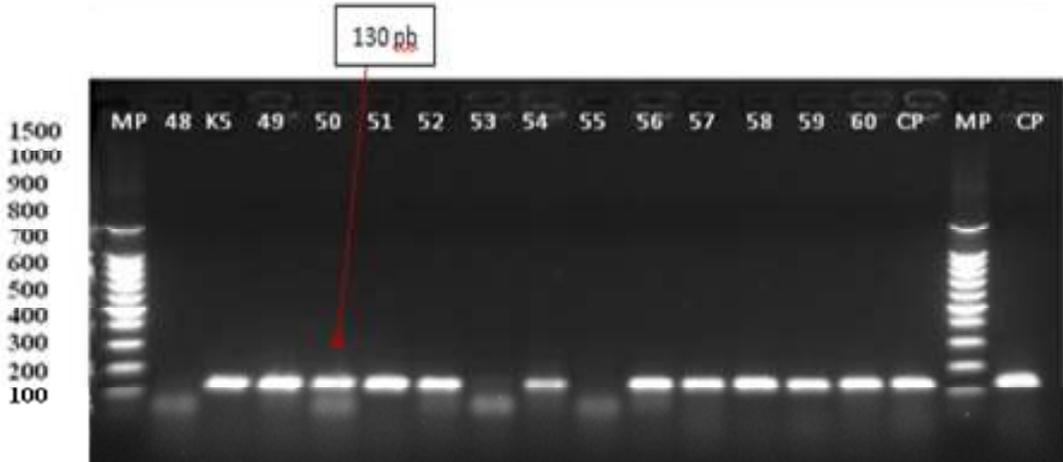


FIGURE 4: STRAINS 53 AND 55 CONTAIN WATER FOR INJECTABLE PREPARATION

MP = Molecular weight marker, wells CP, CPK and K5 contain amplicons of *K pneumoniae* already confirmed by PCR = positive controls and wells 6 and 31 contain water for injectable preparation = Negative controls

## DISCUSSION

Most gave a band for the *K. pneumoniae* 16S-23S internal transcribed spacer region. Amplification of the 16S rRNA gene represents a highly accurate and versatile method for the identification of bacteria to the species level, even when the species in question is notoriously difficult to identify by biochemical methods (17). The K1 serotype was not found in our study. It is disagreed with results elucidated by other workers (15,16) who noticed that serotypes K1, K2 and Non-K1/K2 accounted for 14.3 % (7/49), 38.8 % (19/49) and 46.9 % (23/49) of all *K. pneumoniae* isolates, respectively. Our results were in agreement with those who reported that *K. pneumoniae* serotype K1 is dominant on the other serotypes and find K1 and serotype K2 was 52.3% and 22.7% (18 19) .

This could be related to the isolation site of our strains, more than 70% are non-invasive strains. Otherwise the capsular serotype K1 is recognized as the most virulent and the most encountered throughout the world especially in the countries of Asia where it is correlated to hepatic abscesses (17). In a study of strains of *K. pneumoniae* from 11 Asian countries serotype K1 are findings were 27,5%, 12,6% and 9,6% in Taiwan, Korea and Vietnam respectively. Although cases have been reported in South Africa and Nigeria, no case of Liver Abscesses has been described in our context. This could explain the absence of serotype K1 in our series. But the absence of serotype K1 could also be related to the size of our sample. *K. pneumoniae* serotype K2, it was found in (4/51)7.8% of the strains.

Our results were in agreement with some workers (18,19,20) who reported that *K. pneumoniae* serotype K2 is dominant (64%) on the over serotypes of the three serotypes K1 K2 et K5 researched. Serotype K2 is one of the most common and most invasive capsular serotypes described

## REFERENCES BIBLIOGRAPHIQUES

1. Vardakas, K. Z., Matthaiou, D. K., Falagas, M. E., Antypa, E., Koteli, A., & Antoniadou, E. (2015). Characteristics, risk factors and outcomes of carbapenem-resistant *Klebsiella pneumoniae* infections in the intensive care unit. *Journal of Infection*, 70(6), 592-599.
2. Cortés, G., Borrell, N., de Astorza, B., Gómez, C., Sauleda, J., & Alberti, S. (2002). Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *Klebsiella pneumoniae* in a murine model of pneumonia. *Infection and immunity*, 70(5), 2583-2590.
3. Lin, Y. T., Wang, Y. P., Wang, F. D., & Fung, C. P. (2015). Community-onset *Klebsiella pneumoniae pneumonia* in Taiwan: clinical features of the

throughout the world. A similarly wide range of capsular serotypes has been demonstrated in other studies. However, there are differences in the serotypes that appear most frequently in some countries. Non-K1/K2 strains constituted a very important proportion of the strains of our study with more than 90%. Our results were in agreement with Adam et al. (2006)(21) in Australia who noticed high prevalence of non K1/K2 strains in 96% of 293 strains. They are by far from Lin et al. (2010)(22,) who also found in their series a predominance of non-K1 / K2 serotypes (46.9%). However, they remain discordant with those of many Asian authors in whom serotypes K1 and K2 are predominant (23). In general, there is a variable global distribution of *Klebsiella* capsular serotypes

The *rmpA* gene is the regulator of capsular synthesis; many studies have suggested that this gene could be responsible for the hypervirulent phenotype of *K. pneumoniae* characterized by the hypervirulent character of the strains and found the gene more often associated with serotypes K2 than K1 and non-K1 / K2 serotypes (24). This gene was not found in our study

## Conclusion

Capsular serotypes Non-K1/K2 were the most recovered hence the interest of more studies in order to identify them. Moreover, the determinants of hyper virulence were not found despite the presence of strains positive to the string test.

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disease and associated microbiological characteristics of isolates from pneumonia and nasopharynx. *Frontiers in microbiology*, 6, 122.

4. Fung, C. P., Chang, F. Y., Lee, S. C., Hu, B. S., Kuo, B. I., Liu, C. Y., & Siu, L. K. (2002). A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis?. *Gut*, 50(3), 420-424.
5. Siu, L. K., Yeh, K. M., Lin, J. C., Fung, C. P., & Chang, F. Y. (2012). *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. *The Lancet infectious diseases*, 12(11), 881-887
6. Ohmori, S., Shiraki, K., Ito, K., Inoue, H., Ito, T., Sakai, T., ... & Nakano, T. (2002). Septic endophthalmitis and meningitis associated with *Klebsiella pneumoniae* liver abscess. *Hepatology research*, 22(4), 307-312.

7. Rahimian, J., Wilson, T., Oram, V., & Holzman, R. S. (2004). Pyogenic liver abscess: recent trends in etiology and mortality. *Clinical infectious diseases*, 39(11), 1654-1659.
8. Fang, C. T., Chuang, Y. P., Shun, C. T., Chang, S. C., & Wang, J. T. (2004). A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. *The Journal of experimental medicine*, 199(5), 697-705.
9. Yeh, K. M., Lin, J. C., Yin, F. Y., Fung, C. P., Hung, H. C., Siu, L. K., & Chang, F. Y. (2010). Revisiting the importance of virulence determinant magA and its surrounding genes in *Klebsiella pneumoniae* causing pyogenic liver abscesses: exact role in serotype K1 capsular formation. *Journal of Infectious Diseases*, 201(8), 1259-1267.
10. Shon, A. S., Bajwa, R. P., & Russo, T. A. (2013). Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. *Virulence*, 4(2), 107-118.
11. Meité S, C Boni-cisse, P Monemo, C J Babo, AP Mlan Tanoa, H Faye-Kette, M Dosso, A Amonkou. (2011) Profil de résistance des bactéries isolées des prélèvements bronchiques protégés chez des patients sous ventilation assistée en réanimation CHU de Yopougon. *Méd Afr Noire*, 2011, 58 (8/9) :416-422.
12. C Boni-Cissé, Meite S, N D Coulibaly, N Guessend AP Mlan, H Faye-Kette, M Dosso, KM Timite (2012) Prévalence des gènes qnr chez *Klebsiella pneumoniae* isolés dans le service de pédiatrie du CHU de Yopougon à Abidjan Côte d'Ivoire. *Rev int sc méd*. 2012 ;14(2) :66-72.
13. Meité S, Boni-cissé C. Monemo P, Mlan Tanoa AP, Faye-Ketté H, Dosso M. (2011) Surveillance microbiologique des surfaces au niveau d'un établissement hospitalier de niveau tertiaire : exemple du CHU de Yopougon, Abidjan, Côte d'Ivoire. *J. sci pharm Biol*, 2010, 11(1) :73-81.
14. Turton, J. F., Baklan, H., Siu, L. K., Kaufmann, M. E., & Pitt, T. L. (2008). Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella sp.* and comparison of isolates within these serotypes. *FEMS microbiology letters*, 284(2), 247-252
15. Nadasy, K. A., Domiati-Saad, R., & Tribble, M. A. (2007). Invasive *Klebsiella pneumoniae* syndrome in North America. *Clinical Infectious Diseases*, 45(3), e25-e28
16. Liu, Y., Liu, C., Zheng, W., Zhang, X., Yu, J., Gao, Q., & Huang, X. (2008). PCR detection of *Klebsiella pneumoniae* in infant formula based on 16S-23S internal transcribed spacer. *International journal of food microbiology*, 125(3), 230-235
17. Turton, J. F., Perry, C., Elgohari, S., & Hampton, C. V. (2010). PCR characterization and typing of *Klebsiella pneumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. *Journal of medical microbiology*, 59(5), 541-547.
18. Victor, L. Y. (2007). Virulence characteristics of *Klebsiella* and clinical manifestations of *K. pneumoniae* bloodstream infections. *Emerging Infectious Disease*, 13(7), 123-128.
19. Majid H. Al-Jailawi , Tamara H. Zedan , Kifah A. Jassim. ( 2014). Multiplex-PCR Assay for Identification of *Klebsiella pneumoniae* Int. J. Pharm. Sci. Rev. Res., 26(1),112-117
20. Chung, D. R., Park, M. H., Kim, S. H., Ko, K. S., Kang, C. I., Peck, K. R., & Song, J. H. (2012). Prevalence and molecular characterization of serotype K1 *Klebsiella pneumoniae* strains from various clinical specimen sources in 11 Asian countries. *Journal of Infection*, 64(6), 622-625.
21. Chang, W. N., Huang, C. R., Lu, C. H., & Chien, C. C. (2012). Adult *Klebsiella pneumoniae* meningitis in Taiwan: an overview. *Acta Neurol Taiwan*, 21(2), 87-96.
22. Lin, Y. T., Jeng, Y. Y., Chen, T. L., & Fung, C. P. (2010). Bacteremic community-acquired pneumonia due to *Klebsiella pneumoniae*: clinical and microbiological characteristics in Taiwan, 2001-2008. *BMC infectious diseases*, 10(1), 1.
23. Chang, S. C., Fang, C. T., Hsueh, P. R., Chen, Y. C., & Luh, K. T. (2000). *Klebsiella pneumoniae* isolates causing liver abscess in Taiwan. *Diagnostic microbiology and infectious disease*, 37(4), 279-284.
24. Majid H. Al-Jailawi , Tamara H. Zedan , Kifah A. Jassim. ( 2014). Multiplex-PCR Assay for Identification of *Klebsiella pneumoniae* Int. J. Pharm. Sci. Rev. Res., 26(1),112-117