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Acinetobacter baumannii infections among patients in intensive care unit at the University College Hospital, Ibadan, Nigeria

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Abstract:

Background: Healthcare associated infections (HAIs), also known as nosocomial infections, pose a major safety risk for both patients and healthcare workers globally. *Acinetobacter* species are Gram-negative bacteria primarily associated with HAIs and have been implicated in outbreaks of infections such as ventilator-associated pneumonia, urinary tract infections, and septicaemia. The multi-drug resistance (MDR) nature of *Acinetobacter* species coupled with ability to survive for long periods in harsh conditions have made them of global health concern. Patients in intensive care units (ICUs) are highly susceptible to *Acinetobacter* infection. This study was designed to determine the prevalence and antibiotic resistance profiles of *Acinetobacter* isolates in infections among patients in the ICU of the University College Hospital, Ibadan, Nigeria.
Methodology: A total of 120 consecutive patients were recruited into this study between April and December 2021 from whom blood samples, tracheal aspirates and catheter urine samples were obtained. Samples were obtained from patients who had spent a minimum of 48 hours in the ICU at the time of sample collection. The samples were cultured, and bacterial isolates were identified using standard microbiological methods. Antibiotic susceptibility testing was performed on all isolates by the disc diffusion method in line with the Clinical and Laboratory Standards Institute (CLSI) guideline. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0.
Results: From the samples obtained from 120 enrolled patients during the study period, only 5 were positive for *Acinetobacter* species, giving a prevalence of 4.2%. Four (80.0%) of the isolates were *Acinetobacter baumannii* while 1 (20.0%) was *Acinetobacter haemolyticus.* Four (80.0%) isolates were from the tracheal aspirates, 1 (20.0%) was from the blood culture, while the catheter urine specimen yielded no *Acinetobacter* isolate. The isolates were 80.0% resistant to amikacin, 60.0% to gentamicin, 60.0% to ceftazidime, 40.0% to ciprofloxacin, 40.0% to levofloxacin, 40.0% to meropenem, and 0% to colistin. The 4 *A. baumannii* isolates were multi-drug resistant (resistant to three or more classes of antibiotics) while the only *A. haemolyticus* was mono-drug resistant.
Conclusion: The need for continued infection prevention and control, surveillance of multi-drug resistant *Acinetobacter* spp and antimicrobial stewardship practices in the ICU is hereby emphasized.

Keywords: *Acinetobacter baumannii*, intensive care unit, multi-drug resistance

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Infections à *Acinetobacter baumannii* chez les patients de l'unité de soins intensifs de l'Hôpital Universitaire d'Ibadan, Nigéria

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Résumé:

Contexte: Les infections associées aux soins (IAS), également appelées infections nosocomiales, représentent

un risque majeur pour la sécurité des patients et des professionnels de santé dans le monde entier. Les espèces d'*Acinetobacter* sont des bactéries à Gram négatif principalement associées aux IAS et ont été impliquées dans des épidémies d'infections telles que la pneumonie acquise sous ventilation mécanique, les infections urinaires et la septicémie. La multirésistance aux médicaments (MDR) des espèces d'*Acinetobacter*, associée à leur capacité à survivre longtemps dans des conditions difficiles, en fait une préoccupation sanitaire mondiale. Les patients en unité de soins intensifs (USI) sont très sensibles aux infections à *Acinetobacter*. Cette étude visait à déterminer la prévalence et les profils de résistance aux antibiotiques des isolats d'*Acinetobacter* lors d'infections chez les patients en USI de l' Hôpital Universitaire d'Ibadan, Nigéria.
Méthodologie: Au total, 120 patients consécutifs ont été recrutés pour cette étude entre avril et décembre 2021. Des échantillons de sang, d'aspirations trachéales et d'urine ont été prélevés par cathéter. Français Des échantillons ont été obtenus auprès de patients ayant passé au moins 48 heures en USI au moment du prélèvement. Les échantillons ont été mis en culture et les isolats bactériens ont été identifiés à l'aide de méthodes microbiologiques standard. Un test de sensibilité aux antibiotiques a été effectué sur tous les isolats par la méthode de diffusion sur disque, conformément aux directives de l'Institut Normes Cliniques et de Laboratoire (CLSI). Les données ont été analysées à l'aide du logiciel statistique pour les sciences sociales (SPSS) version 26.0.
Résultats: Parmi les échantillons obtenus auprès de 120 patients inclus pendant la période d'étude, seuls 5 étaient positifs pour les espèces d'*Acinetobacter*, soit une prévalence de 4,2%. Quatre (80,0%) des isolats étaient *Acinetobacter baumannii* tandis qu'un (20,0%) était *Acinetobacter haemolyticus*. Quatre (80,0%) isolats provenaient des aspirats trachéaux, un (20,0%) provenait de l'hémoculture, tandis que l'échantillon d'urine du cathéter n'a donné aucun isolat *Acinetobacter*. Français Les isolats étaient résistants à 80,0% à l'amikacine, à 60,0% à la gentamicine, à 60,0% à la céftazidime, à 40,0% à la ciprofloxacine, à 40,0% à la lévofloxacine, à 40,0% au méropénème et à 0% à la colistine. Les 4 isolats d'*A. baumannii* étaient multirésistants (résistants à trois classes d'antibiotiques ou plus) tandis que le seul *A. haemolyticus* était monorésistant.
Conclusion: La nécessité d'une prévention et d'un contrôle continus des infections, d'une surveillance des espèces d'Acinetobacter multirésistantes et de pratiques de gestion des antimicrobiens dans l'unité de soins intensifs est ici soulignée.

Mots-clés: *Acinetobacter baumannii,* unité de soins intensifs, multirésistance

Introduction:

Infection is a frequent and major cause of morbidity and mortality in the intensive care units (ICU) (1,2). *Acinetobacter* is one of the important Gram-negative pathogens causing serious healthcare-associated infections (HAIs) and this is a major public health concern (3,4). *Acinetobacter baumannii* is reportedly ubiquitous, responsible for serious infections in hospitalized patients, with a high propensity to develop resistance to antimicrobial agents (5). Vincent et al., (6) reported that HAIs affect about 5% to 15% of hospitalised patients on regular wards and can have a rate of 50.0% or more in patients in intensive care units. *Acinetobacter baumannii* has been reported in ventilator associated pneumonia with high morbidity and mortality (6).

Acinetobacter spp are considered as part of normal flora of the skin, mucosa of the pharynx and the human respiratory secretions (7). Furthermore, *A. baumannii* is frequently isolated from reusable medical equipment such as ventilator tubings, monitoring devices, humidifiers and respirometers as documented by Kanafani & Kary (8). According to Odewale et al., (9)*, Acinetobacter* is known to persist and survive on surfaces under a wide variety of environmental conditions, therefore it is a common cause of outbreak.

The study by Ayobami et al., (10) showed that there is evidence of increasing *A. baumannii* outbreaks in healthcare facilities across sub-Saharan Africa and Eastern Mediterranean countries. *Acinetobacter baumannii* strains have been identified to have acquired multiple drug resistance and are isolated or identified in numerous hospitals throughout the world in studies by Abbo et al., (11) and Kanafani and Kary (8). High prevalence of *Acinetobacter* infection has been reported worldwide, 19.2% in Asia, 17.1% in Eastern Europe, 14.8% in Africa, 13.8% in Central and South America (12). Therefore, frequent review of antibiotic susceptibility pattern of clinical location specific isolates is necessary for proper prevention and control of healthcare associated infections and hospital acquired outbreaks.

The general objective of this study was to determine the prevalence and antimicrobial susceptibility of *A. baumannii* isolates among patients in the intensive care unit (ICU) of the University College Hospital (UCH), Ibadan, Nigeria. The specific objectives are to determine the prevalence of *A. baumannii* infections among ICU patients of the hospital, evaluate the distribution of *Acinetobacter* spp in the different specimen obtained from these patients and determine the antimicrobial susceptibility and resistance profiles of the isolates from these patients.

Materials and method:

Study design and setting:

This was a descriptive, cross-sectional study involving patients who had spent at least 48 hours in the ICU of the hospital as at the time of data collection. The study was carried out in the department of medical microbiology and the ICU, UCH, Ibadan, Nigeria from April 1, 2021 to September 30, 2021. The ICU is a 12-bed facility, with an average monthly admission of 30-40 patients.

Study participants:

The study participants were patients, who at the time of data collection, had been on admission at the ICU for up to or more than 48 hours and included those on mechanical ventilation and those who had urinary catheter *in situ.* The reasons for ICU admission from the Emergency Department included pneumonia, respiratory failure and sepsis while respiratory failure was the reason for admission from the general ward. Postoperative admissions into the ICU were due to respiratory insufficiency and/or cardiovascular instability. Patients who had been on ICU admission less than 48 hours and those with poor condition or *in-extremis* were excluded.

Determination of sample size and sampling method:

The minimum sample size was determined using the Fisher's formula; $n=z^2(1-p)p/d^2$, where n is minimum sample size, p is prevalence of 8.5% in a previous study (9), z is 1.96, and d is tolerance/error of 5%. This gave a calculated minimum sample size of 120. A random sampling technique was used to select the participants included in this study. Consecutive admission into the ICU from the start of the study, that met inclusion criteria until the minimal sample size was achieved at the end of the period.

Specimen collection:

Tracheal aspirate, blood and catheter urine specimens were obtained by aseptic procedure from the patients and for each specimen. For blood culture, about 5ml of venous blood was obtained from each patient, into a BD BACTEC aerobic blood culture bottle for incubation. Tracheal aspirate was obtained using sterile mucus extractor connected to the suction machine. Aspirate was then transferred into a sterile universal bottle and immediately transported to the microbiology laboratory for further processing.

For catheter urine, the urethral catheter was disconnected from the urine bag and clamped for 10-20 minutes after which the urine collecting in the catheter was allowed to drip into a sterile universal bottle. All biological samples were promptly sent to the microbiology laboratory, for microscopy, culture and sensitivity testing.

Specimen culture for *Acinetobacter* isolation:

Blood, catheter urine and tracheal aspirate samples were inoculated on MacConkey agar (Mac, Oxoid) and Chocolate agar plates and incubated for 18-24 hours at 35-37^{0}C and examined for bacterial growth. All non-lactose fermenters on MacConkey agar plate were subcultured to obtain pure isolates.

Phenotypic identification of *Acinetobacter* isolates:

Identification of *Acinetobacter* isolate was achieved by examination of colony morphology on culture plates, Gram staining, and biochemical tests which included citrate, catalase, indole and oxidase tests. Gram negative coccobacilli that were non-lactose fermenting, citrate and catalase positive, and oxidase and indole negative were presumptively considered as *Acinetobacter baumannii*. The Microbact 24E (MB24E) system was used to phenotypically confirm *Acinetobacter baumannii* following approved procedure described by Ling et al., (13).

Antimicrobial susceptibility test:

Antimicrobial susceptibility test (AST) was performed by the modified Kirby-Bauer test disc diffusion method. The inoculum was prepared as a suspension of the isolate by picking 2 or 3 colonies of the isolate with a sterile straight wire and making an emulsion of it in peptone water. This suspension was standardized by comparing it with 0.5 McFarland turbidity standard.

Using a sterile swab stick, sterile Mueller-Hinton (MH) agar plates were inoculated with the broth cultures. After about 3 minutes, antibiotic-impregnated discs (amoxicillin-clavulanic acid 30µg, amikacin 30µg, meropenem 10µg, ciprofloxacin 5µg, gentamicin 10µg, levofloxacin 5µg, ceftazidime 30µg, colistin 10 µg) were placed on the surface of the agar and incubated at 35-37^{o}C for 24 hours.

The diameter of the zones of inhibition were measured with a standard calibrated meter rule and interpreted as sensitive or resistant in line with standard interpretative CLSI charts as described by Benkova et al., (14).

Statistical analysis:

Data were entered into Microsoft Excel and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26. Descriptive statistics were employed to summarize the data. Variables such as age group, source of admission, type of biological samples, and antimicrobial susceptibility patterns were summarized using frequencies and percentages. Chi square was performed to examine associations between categorical variables. A significance level of $p<0.05$ was considered statistically significant.

Results:

Characteristics of the participants:

The age of the 120 participants ranged from 8 to 82 years, with 94 (78.3%) females and 26 (21.7%) males. Ninety-nine patients (82.5%) were admitted from the Accident and Emergency department while 11 (9.2%) and 10 (8.3%) were from the general wards and operating theatres of the hospital respectively (Table 1). Blood samples were collected from

40 (33.3%) of the participants, tracheal aspirates from 36 (30.0%) and catheter urine from 44 (36.7%) (Table 1).

Prevalence of *Acinetobacter* infection:

Acinetobacter species were isolated from samples of 5 participants (4 *A. baumannii* and 1 *A. haemolyticus*), giving a prevalence of 4.2%, with 1 (2.5%) isolate from the 40 blood culture samples, 4 (11.1%) isolates from the 36 trachea aspirates, and none (0%) from the 44 urine samples.

The prevalence of *Acinetobacter* isolation was not significantly associated with age or sex of participants (p>0.05) but was significantly associated with site of isolation, with significantly higher rate of isolation from respiratory tract (11.1%) than from blood (2.5%) and urinary tract (0%) (p=0.038) (Table 2).

Table 1: Characteristics of participants

Characteristics	Frequency	Percentage
Age group (years)		
<10	2	1.7
10 – 29	26	21.7
30 – 39	45	37.5
40 – 49	31	25.8
50 – 59	5	4.2
≥ 60	11	9.2
Sex		
Males	94	78.3
Females	26	21.7
Admission sources		
Accident & Emergency	99	82.5
General ward	11	9.2
Operating theatre	10	8.3
Biological samples		
Blood	40	33.3
Tracheal aspirate	36	30.0
Catheter urine	44	36.7
Total	**120**	**100.0**

Table 2: Prevalence of *Acinetobacter* infection by age, sex and site of isolation

Variables	No of patients	No infected with *Acinetobacter*	Percentage	x^2	p value
Age group (years)					
<10	2	0	0	1.203	0.9446^{ns}
10 – 29	26	1	12.5		
30 – 39	45	2	4.8		
40 – 49	31	2	6.4		
50 – 59	5	0	0		
≥ 60	11	0	0		
Sex					
Male	94	5	5.3	0.4184	0.5177^{ns}
Female	26	0	0		
Site of isolation					
Blood	40	1	2.5	6.539	0.038^{s}
Respiratory	36	4	11.1		
Urinary	44	0	0		
Total	**120**	**5**	**4.2**		

ns = not statistically significant; s = statistically significant (p<0.05)

Table 3: Antimicrobial susceptibility and resistance of *Acinetobacter* isolates

Antibiotics	Gen	Amk	Cef	Lev	Cipro	Mer	Col
Isolate 1	R	R	R	R	S	S	S
Isolate 2	R	R	R	S	R	S	S
Isolate 3	R	R	S	S	S	R	S
Isolate 4	S	R	R	S	R	S	S
Isolate 5	S	S	S	S	S	R	S
% Resistant	**60.0**	**80.0**	**60.0**	**20.0**	**40.0**	**40.0**	**0**

R = Resistant; Isolates 1 – 4: *A baumannii;* Isolate 5: *A. haemolyticus;* G Gen = Gentamicin; Amk = Amikacin; Ceft = Ceftazidime; Lev = Levofloxacin; Cipro = Ciprofloxacin; Mer = Meropenem, Col = Colistin

Table 4: Antimicrobial resistant profiles of *Acinetobacter* isolates

Isolates number	Isolate	Resistant profile	
1	*Acinetobacter baumannii*	$Gen^R/Amk^R/Cef^R/ /Lev^R$	MDR (3)
2	*Acinetobacter baumannii*	$Gen^R/Amk^R/Cef^R/Cipr^R$	MDR (3)
3	*Acinetobacter baumannii*	$Gen^R/Amk^R/Mer^R$	MDR (3)
4	*Acinetobacter baumannii*	$Amk^R/Cef^R/Cip^R$	MDR (3)
5	*Acinetobacter haemolyticus*	Mer^R	Mono-resistant

MDR = Multi-drug resistant; Gen = Gentamicin; Amk = Amikacin; Ceft = Ceftazidime; Lev = Levofloxacin; Cipro = Ciprofloxacin; Mer = Meropenem

Antimicrobial susceptibility of *Acinetobacter* isolates:

All (100%) the *Acinetobacter* isolates were susceptible to colistin, 4 (80.0%) isolates were resistant to amikacin, 3 (60.0%) isolates were resistant to gentamicin and ceftazidime, 2 (40.0%) to ciprofloxacin and meropenem, and 1 (20.0%) isolate was resistant to levofloxacin (Table 3). Except for *A. haemolyticus*, all the *A baumannii* isolates were multi-drug resistant (Table 4).

Discussion:

This study was undertaken to determine the prevalence of *Acinetobacter baumannii* among patients admitted to the intensive care unit of the University College Hospital Ibadan, a tertiary health facility in Oyo State of Nigeria. The prevalence of *Acinetobacter* spp in ICU in this study was 4.17%. This was lower than a previously reported prevalence of 79.0% in the same hospital in 2014 by Nwadike et al., (15). This could be due to difference in sample size as Nwadike et al had a sample size of 400 as against 120 samples in this study.

Different hospital prevalence of *A. baumannii* has been reported in many countries. In Kenya, 22.7% prevalence was reported (1), Ethiopia reported 2.5% (3), Tamil Nadu/India reported 11.17% (16), Honduras 34.5% (5) while in Saudi-Arabia, prevalence of 8.3% was reported (8) and 5.4% in Ibadan, Nigeria (17). These prevalence reports support the fact that *A. baumannii* is not only ubiquitous but worldwide. The details of the context and specimen sample however reveal the complexity of these reports. Specimen/sample for analysis included blood, urine, tracheal aspirate, sputum, surgical wound swab and the environment hence the wide variation in prevalence rates.

In this study, the prevalence of *Acinetobacter* isolation was 2.5% from blood, 11.1% from tracheal aspirate and 0% from urine. In Kenya (1), 7% was from the blood or tracheal aspirate and 12.1% from urine sample. A study from Dhaka, Bangladesh (4) reported 10.7% from the blood, 68% from respiratory samples and 40.3% from the urine, while the study from Ibadan, Nigeria (15), reported prevalence of 7% from blood, 7% from urine, and 86% from tracheal aspirates. These studies like ours showed higher prevalence of *Acinetobacter* isolation from tracheal/respiratory samples. This specimen-specific prevalence may suggest areas where the need exist for preventive interventions.

While prevalence deduced from systematic reviews and meta-analysis give a long-term measure of infection, country-wide or hospital-wide prevalence gives the impression of geographical and location-specific prevalence and challenge. The prevalence in specimens of catheter urine, tracheal aspirate and blood samples however suggest the risk posed by invasive techniques, ineffective/inadequate aseptic technique and instrumentation especially in ICU patients with lowered immunity. These need careful consideration and policy.

All the *A. baumannii* isolates from ICU patients in this study were from males. Garcia-Garmendia et al., (18) in their study posited that the male gender is at a higher risk of *A. baumannii* infections and reported a male: female ratio of 9:1. Also, gender disparity in *A. baumannii* infection was reported by Iregbu et al., (19) with 1.9: 1 male: female ratio from age 45 years and above. The age range of the patients in our study was 8 to 82 years. However, 4 (80.0%) of the isolates were recovered from male participants aged 30-49 years while 1 (20.0%) was from a participant in the 10-19 years age group. This age-group was lower than that reported by Odewale et al., (9).

The highest rates of infections were reported in the elderly patients by Al Samawi et al., (20) and Nwadike et al., (15). A wider age-range was reported by Unwigabiye et al., (21). Advancing age with several co-morbidities and lowered immunity may partially account for these findings. While our findings were closer to that of Mushtaqa et al., (22) who reported 6.2% in Pakistan, it is lower than that reported by Odewale et al., (9), in a hospital-wide study. The study by Nwadike et al., (15) ten years earlier in the same location as our study, reported a higher prevalence at the time of their study. Many factors interplay to determine prevalence of infection and could not have been due to time-trend alone. An improvement in infection control being observed in the hospital may be contributory.

Four of the *A. baumannii* were isolated from tracheal aspirates while only one from

blood samples and none from catheter urine. This is contrary to the findings by Dada-Adegbola et al (16) in a hospital-wide sampling where 41.0% of *Acinetobacter* species were isolated from blood samples, and 24.0% from wound swabs. Several studies have reported that respiratory tract produce the highest rate of *A. baumannii* infection as reported by Sileem et al., (23), Mathai et al., (24), and Patel et al., (25). A major risk factor for *A. baumannii* respiratory tract infection is mechanical ventilation and its duration; the longer a patient is on mechanical ventilation, the higher the risk of developing hospital-acquired ventilator-associated infection (6).

Acinetobacter species have shown increasing resistance to major antibiotic classes including β-lactams, aminoglycosides, and fluoroquinolones, hence, the World Health Organization (WHO) has included *A. baumannii* on the list of pathogens that should be given priority in the Research and Development of new antibiotics (26). Savov et al., (27), Mathai et al., (24) and Odewale et al., (9) all reported resistance of *A. baumannii* to currently known antibiotics (ampicillin, cephalothin, carbenicillin, gentamicin, amikacin, chloramphenicol, tetracycline, cephalosporins), therefore presenting a therapeutic dilemma for infection management the world over. In our study, all the isolates were susceptible to colistin, despite the multi-drug resistance to at least three other groups of antimicrobials. Increased susceptibility of *A. baumannii* isolates to colistin have been reported by Zafer et al., (28) and Odewale et al., (9). But for its toxicity, colistin alone or in combination therapy has been recommended for treatment of MDR *A. baumannii* infection.

Several interventions for *A. baumannii* infection control have been practiced. One such intervention is bacteriological surveillance in hospitals with daily review of antibiotic susceptibility of all clinical isolates as suggested by Urban et al., (29) and Mehtap et al., (30). Antibiotic stewardship committee has been suggested by Nwadike et al., (15) as well as adherence to and evaluation of infection prevention and control by Kipsang et al., (1). Finally providing adequate training periodically to hospital staff, observance of aseptic techniques during any medical procedure in addition to routine antimicrobial susceptibility test will reduce *A. baumannii* and other infective agents according to Kamal et al., (3).

The limitations of this study include the small number of patients who had *Acinetobacter* infection. This calls for caution in interpreting and extrapolating the results. Secondly, the phenotypic method used for identification of species is not as sensitive as the molecular technique which can both identify and detect the genetic basis of resistance in the isolates.

Conclusion:

The multi-drug resistant nature of *Acinetobacter* spp poses therapeutic challenge to the clinicians and patients. Antimicrobial stewardship and infection prevention control are critical measures to reduce the prevalence of *A. baumannii* (and other MDR pathogens) infections in the intensive care unit.

Contributions of authors:

OOA, OOO, and SAA contributed to the conceptualization and study design. OOO and SAA contributed to data collection. The laboratory analysis was performed by OOA and OOO. All authors contributed to the statistical analysis, manuscript writing, and approval of the final manuscript submitted for publication.

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Authors declare no conflict of interest

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