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Laboratory survey of extended spectrum beta-lactamase producing enterobacteriaceae in clinical infections among hospitalised patients at LAUTECH Teaching Hospital, Ogbomoso, Nigeria

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Abstract:

Background: The extended-spectrum beta-lactamase (ESBL) producing enterobacteriaceae are a major public health threat globally, causing both community and healthcare associated infections (HAIs). Due to multi-resistant nature of these strains, infections caused by them are associated with treatment failure, high mortality, and increased healthcare costs. This laboratory survey determined the prevalence of infections caused by ESBL-producing enterobacteriaceae in LAUTECH Teaching Hospital, Ogbomoso

Methodology: Over three years (January 2016 to December 2018), non-duplicate clinical samples (sputum, blood, urine, and wound swabs) collected from hospitalised patients with suspected clinical infections were routinely processed at microbiology laboratory of our hospital for aerobic culture and isolation of enterobacteriaceae. Antibiotic susceptibility of each isolate to routinely used antibiotics was determined by the disk diffusion method and 'double disk' synergy test was used to routinely confirm ESBL production. Demographic and clinical data were extracted from the requisition form.

Results: Of the total 4,198 hospitalised patients over the three year period, 1,222 (29.1%) had clinical infections, out of which 689 (16.4%) were laboratory confirmed. Enterobacteriaceae were isolated from 343 patients (prevalence rate, 8.2%) while ESBL producers were isolated from 46 (prevalence rate, 1.1%). The most frequent enterobacteriaceae were *Klebsiella* spp (54.5%) and *Escherichia coli* (35.9%) recovered mainly from urinary tract infection (UTI, 45.2%), skin and soft tissue infection (SSTI, 27.9%) and lower respiratory tract infection (LRTI, 17.5%) but ESBL producers were frequently associated with osteomyelitis (50%), LRTI (18.3%) and SSTI (14.6%). The ESBL producers were all resistant to ampicillin, cefotaxime, ceftazidime, cefepime, gentamicin, and ciprofloxacin but susceptible to imipenem. The non-ESBL producers were comparatively less resistant with 43.8%, 34.3%, 29%, 35%, 43%, 37%, and 4% resistant to ampicillin, cefotaxime, ceftazidime, cefepime, gentamicin, ciprofloxacin and imipenem respectively.

Conclusion: The prevalence of clinical infections among hospitalised patients in our facility is high but the rate of ESBL-producing enterobacteriaceae is relatively low. In spite of this, there is need for continuous surveillance of ESBL and other antibiotic resistant pathogens as part of the infection prevention and control (IPC) programme, with implementation of measures that will reduce the incidence of these infections in our hospital.

Keywords: Laboratory survey; hospitalised patients; ESBL; multidrug resistance

Enquête de laboratoire sur les entérobactéries productrices de bêta-lactamases à spectre étendu lors d'infections cliniques chez des patients hospitalisés à l'hôpital universitaire LAUTECH, à Ogbomoso, au Nigéria

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Abstrait:

Contexte: Les entérobactéries productrices de bêta-lactamase à spectre étendu (BLSE) constituent une menace majeure pour la santé publique dans le monde, provoquant à la fois des infections dans la communauté et des infections associées aux soins de santé. En raison de la nature multirésistante de ces souches, les infections qu'elles provoquent sont associées à un échec du traitement, à une mortalité élevée et à une augmentation des coûts de soins de santé. Cette enquête en laboratoire a permis de déterminer la prévalence d'infections causées par des entérobactéries productrices de BLSE à l'hôpital universitaire LAUTECH, à Ogbomoso.

Méthodologie: Sur trois ans (janvier 2016 à décembre 2018), des échantillons cliniques non dupliqués (expectorations, sang, urine, et plaies oreilles) prélevés chez des patients hospitalisés présentant des suspicions d'infections cliniques ont été systématiquement traités dans le laboratoire de microbiologie de notre hôpital pour culture aérobie et isolement d'entérobactériacées. La sensibilité aux antibiotiques de chaque isolat aux antibiotiques utilisés en routine a été déterminée par la méthode de diffusion sur disque et un test de synergie «double disque» a été utilisé pour confirmer en routine la production de BLSE. Les données démographiques et cliniques ont été extraites du formulaire de demande.

Résultats: Sur un total de 4198 patients hospitalisés au cours de la période de trois ans, 1222 (29,1%) ont présenté une infection clinique, dont 689 (16,4%) ont été confirmés en laboratoire. Des entérobactéries ont été isolées chez 343 patients (taux de prévalence de 8,2%), tandis que les producteurs de BLSE ont été isolés chez 46 patients (taux de prévalence de 1,1%). Les entérobactériacées les plus fréquentes étaient *Klebsiella* spp (54,5%) et *Escherichia coli* (35,9%) principalement dues à une infection des voies urinaires (UTI, 45,2%), une infection de la peau et des tissus mous (SSTI, 27,9%) et des voies respiratoires inférieures (LRTI, 17,5%), mais les producteurs de BLSE étaient fréquemment associés à l'ostéomyélite (50%), au LRTI (18,3%) et au SSTI (14,6%). Les producteurs de BLSE étaient tous résistants à l'ampicilline, au céfotaxime, à la ceftazidime, au céfépime, à la gentamicine et à la ciprofloxacine, mais sensibles à l'imipénem. Les producteurs non BLSE étaient comparativement moins résistants, avec respectivement 43,8%, 34,3%, 29%, 35%, 43%, 37% et 4% de résistance à l'ampicilline, au céfotaxime, au ceftazidime, au céfépime, à la gentamicine, à la ciprofloxacine et à l'imipénème.

Conclusion: La prévalence d'infections cliniques chez les patients hospitalisés dans notre établissement est élevée mais le taux d'entérobactéries productrices de BLSE est relativement faible. Malgré cela, il est nécessaire de surveiller en permanence les BLSE et les autres agents pathogènes résistants aux antibiotiques dans le cadre du programme de prévention et de contrôle des infections (IPC), avec la mise en œuvre de mesures permettant de réduire l'incidence de ces infections dans notre hôpital.

Mots clés: Enquête de laboratoire; patients hospitalisés; BLSE; résistance multiple aux médicaments

Introduction:

Infections caused by the extended spectrum beta lactamase (ESBL) producing enterobacteriaceae are a major public health threat globally (1, 2). ESBLs confer resistance to all beta lactam antibiotics except carbapenems and cephamycins (2). They may also confer resistance to additional antibiotic classes such as aminoglycosides, sulfonamides and fluoroquinolones from carriage of plasmids containing resistant genes (3). Infections by these strains are therefore associated with treatment failure, high mortality, and increased healthcare costs (4, 5).

It is important to periodically survey these resistant pathogens in health care institutions for the purpose of infection prevention and control. The objectives of this survey therefore is to identify ESBL producing enterobacteriaceae and determine the prevalence in clinical infections among hospitalized patients in Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Ogbomoso, Nigeria.

Methodology:

Study design and setting

This is a descriptive cross sectional survey of all hospitalised patients with suspected clinical infections at LAUTECH Teaching Hospital, Ogbomoso, during the period January 2016 and December 2018.

Subjects

All patients on admission with fever and other clinical features suggestive of sepsis routinely investigated for microbial aetiology of infections during the study period were eligible.

Specimen and data collection

From each hospitalised patient, non-duplicate clinical specimens such as swabs, sputum, blood or urine as appropriate were collected following recommended guidelines and methods (6). Demographic and clinico-laboratory data were obtained from the patients requisition and laboratory records.

Isolation and susceptibility test

Cell-free culture media appropriate for each sample were inoculated and incubated in aerobic atmosphere at $37^{o}C$ for 24 hours (6). Suspected colonies of enterobacteriaceae on culture media were identified to species level using conventional biochemical test scheme of indole, motility, methyl red, vogues proskauer and citrate utilization (IMMVC) (7).

Invitro antibiotic susceptibility test (AST) of each isolate was determined by the disk diffusion method on Mueller Hinton (MH) agar (8) to routinely used antibiotics (ampicillin 10μg, cefotaxime 30μg, ceftazidime 30μg, cefepime 30μg, gentamicin 10μg, ciprofloxacin 5μg, and imipenem 10μg) in our hospital, and AST results interpreted according to CLSI guideline (9). *Escherichia coli* ATCC 25922 was used as control strain.

ESBL detection

All isolates resistant to cefotaxime or ceftazidime in the AST were tested for ESBL production on MH agar plate using the classical 'double disk' synergy test (10) with amoxicillin (20μg)-clavulanic acid (10μg) disk and cefotaxime (30μg) or ceftazidime (30μg) disk. ESBL production was confirmed when there was greater than 5mm increase in the inhibition zone size produced by the cefotaxime and amoxicillin-clavulanic or ceftazidime and amoxicillin-clavulanic disk over the inhibition zone size produced by the cephalosporin disk alone. *Escherichia coli* ATCC 25922 was used as negative control while *Klebsiella pneumoniae* ATCC 700603 was used as positive control strain.

Statistical analysis

Data were analysed using GraphPad InStat software (San Diego, CA 92105) and presented as frequency distribution tables. Association between categorical variables was measured by Chi square test and $p < 0.05$ was taken as significant value.

Results:

Table 1 shows the prevalence of clinical infections among hospitalised patients in LAUTECH Teaching Hospital, Ogbomoso, during the three year period of survey. Of the total 4,198 patients hospitalised, 1,222 had clinical infections, giving an overall infection prevalence of 29.1% with 24.6% in 2016, 36.4% in 2017 and 29.1% in 2018 ($p<0.0001$).

Bacteria were isolated from samples of 689 (16.4%) hospitalised patients, entero-bacteriaceae from 343 (8.2%) and ESBL producing enterobacteriaceae from 46 (1.1%). The most frequently isolated members of the enterobacteriaceae were *Klebsiella* spp (54.5%), followed by *Escherichia coli* (35.9%), *Proteus* spp (9.3%) and *Enterobacter* spp (0.3%) but ESBL producers were mainly *E. coli* (n=30) and *Klebsiella* spp (n=16) (Table 2).

The clinical infections caused by the enterobacteriaceae included urinary tract infection (UTI, 45.2%), skin and soft tissue infection (SSTI, 27.9%), lower respiratory tract infection (LRTI, 17.5%), blood stream infection (BSI, 2.0%), osteomyelitis (1.7%), and others (5.5%). However, ESBL producing entero-bacteriaceae were frequently associated with osteomyelitis (50%), LRTI (18.3%), SSTI (14.6%), BSI (14.3%) and UTI (10.9%) (Table 3).

The antibiotic susceptibility profile shows that ESBL producing enterobacteriaceae were totally resistant to all tested antibiotics (ampicillin, cefotaxime, ceftazidime, cefepime, gentamicin and ciprofloxacin) but susceptible to imipenem (Table 4). In contrast, the non-ESBL producing enterobacteriaceae showed lower resistance to ampicillin (51.3%), cefotaxime (43.1%), ceftazidime (38.5%), cefepime (34.4%), gentamicin (51.3%) and ciprofloxacin (45.5%) ($p<0.0001$), and only 4% were resistant to imipenem ($p=0.3388$).

Discussion:

The prevalence of clinical infections among hospitalised patients in our hospital is high (prevalence rate of 29.1% over a 3 year period) and this increased significantly in 2017 and 2018 compared to 2016 ($p <0.0001$). This increasing rate may be a pointer to some problems with our infection control practices. The infection prevention and control (IPC) programme of our hospital has not fully taken off and the IPC committee was only officially inaugurated in late 2018 (11). This committee is currently developing IPC plan and policies to address challenges of hospital and community associated infections in our facility.

The prevalence rate of 1.1% among hospitalised patients for clinical infectio n cause

Table 1: Prevalence of clinical infections caused by ESBL producing enterobacteriaceae in LAUTECH Teaching Hospital, Ogbomoso (2016-2018)

Year	No of hospitalized patients	No of clinical infection (*prevalence)	No of clinical infections caused by enterobacteriaceae (*prevalence)	No of clinical infections caused by ESBL producing enterobacteriaceae (*prevalence)		
				Klebs spp	*E. coli*	Total
2016	1747	**430 (24.6%)	177 (10.1%)	14	4	18 (1.0%)
2017	1076	**392 (36.4%)	74 (6.9%)	4	3	7 (0.7%)
2018	1375	**400 (29.1%)	92 (6.7%)	12	9	21 (1.5%)
Total	4198	1222 (29.1%)	343 (8.2%)	30 (0.7%)	16 (0.4%)	46 (1.1%)

*Prevalence of clinical infection was calculated by dividing the number of infection (numerator) by the number of hospitalised patients (denominator);
**Significant difference in infection prevalence between years 2016, 2017 & 2018 (X^2 = 45.005, $p < 0.0001$)

Table 2: Frequency of enterobacteriaceae isolated from patients with clinical infections in LAUTECH Teaching Hospital, Ogbomoso (2016-2018)

Bacteria isolates	No of ESBL producing enterobacteriaceae	No of non-ESBL producing enterobacteriaceae	Total number of enterobacteriaceae (%)
Klebsiella spp	30	157	187 (54.5)
Escherichia coli	16	107	123 (35.9)
Proteus spp	0	32	32 (9.3)
Enterobacter spp	0	1	1 (0.3)
Total	46 (13.4)	297 (86.6)	343 (100)

ESBL = Extended Spectrum Beta Lactamase

Table 3: Clinical infection types and ESBL producing enterobacteriaceae in LAUTECH Teaching Hospital, Ogbomoso (2016-2018)

Clinical infection	No of patients with enterobacteriaceae infection (%) (n=343)	No of patients with ESBL producing enterobacteriaceae infection (%)		
		Escherichia coli (n=16)	*Klebsiella* spp (n=30)	Total (%) (n=46)
Urinary tract infection	155 (45.2)	10	7	17 (10.9)
Skin and soft tissue infection	96 (27.9)	5	9	14 (14.6)
Lower respiratory tract infection	60 (17.5)	0	11	11 (18.3)
Blood stream infection	7 (2.0)	0	1	1 (14.3)
Osteomyelitis	6 (1.7)	1	2	3 (50.0)
*Others	19 (5.5)	0	0	0

ESBL = Extended Spectrum Beta Lactamase; *includes gastroenteritis, ophthalmitis, meningitis

Table 4: *Invitro* antibiotic resistance profiles of enterobacteriaceae isolated from patients with clinical infections in LAUTECH Teaching Hospital, Ogbomoso (2016-2018)

Antibiotics	No of ESBL producers resistant to antibiotics (%) (n=46)	No of non-ESBL producers resistant to antibiotics (%) (n=297)	Total of number of isolates resistant to antibiotics (%) (n=343)	X^2	95% CI	*p* value
Ampicillin	46 (100)	130 (43.8)	176 (51.3)	48.182	0.1985-0.3327	< 0.0001*
Cefotaxime	46 (100)	102 (34.3)	148 (43.1)	67.344	0.2375-0.3925	< 0.0001*
Ceftazidime	46 (100)	86 (29.0)	132 (38.5)	81.944	0.2673-0.4366	< 0.0001*
Cefepime	46 (100)	72 (35.0)	118 (34.4)	97.968	0.3019-0.4834	< 0.0001*
Gentamicin	46 (100)	130 (43.8)	176 (51.3)	48.182	0.1985-0.3327	< 0.0001*
Ciprofloxacin	46 (100)	110 (37.0)	156 (45.5)	61.167	0.2242-0.3734	< 0.0001*
Imipenem	0 (0)	12 (4.0)	12 (3.5)	0.9151	0.02107-0.06947	0.3388**

ESBL = Extended Spectrum Beta Lactamase; X^2 = Chi square; CI = Confidence Interval; * = Statistically significant; ** = Not statistically significant

by ESBL producing enterobacteriaceae in this survey appears relatively low because the rate was calculated in relation to all hospitalised patients (with and without infections) during the survey period, which gives a true reflection of the prevalence in our facility. However, most studies on ESBL producing enterobacteriaceae usually report prevalence among only infected or colonised hospitalised patients, which tends to over-estimate the true prevalence (12-14).

Considering only patients with entero-bacteriaceae infection, Iroha et al., in Nigeria reported 20.3% ESBL rate (12) while Ibrahim et al., in Saudi Arabia reported 27% (14). In the current study, we report a rate of 13.4%, which is similar to 12% rate reported by Obebe et al., (13) in the same geographical region as ours. ESBL colonization or infection of patients in healthcare facility and community have been reported to depend on many factors including antibiotic use/misuse, previous hospitalisation and residence in a long term care facility, use of medical devices, old age, co-morbidity, and adequacy of infection control programmes (15-18).

The ESBL producers in this survey were multiply and highly resistant to the antibiotic disks routinely used in our laboratory (which mirrors commonly prescribed antibiotics in the hospital) compared to the non-ESBL producers with the exception of imipenem to which both groups were highly susceptible. This high resistance rate may have occurred in part from poor antibiotic prescribing practice as antimicrobial stewardship programme is not yet in place in our facility. The misuse/overuse of antibiotics especially the third and fourth generation cephalosporins, fluoroquinolones, and aminoglycosides can select ESBL strains carrying multidrug resistant (MDR) plasmids or integrons (3,19,20), which may be circulating in our facility.

There are a number of limitations to this survey; (i) laboratory surveys have low sensitivity for infection surveillance because positive laboratory result may not indicate infection and negative result does not rule out infection; (ii) the findings of this cross sectional survey have limited application because data were collected only once from infected patients, therefore assessment of infection risk factors could not be done; and (iii) the study design did not differentiate healthcare from community associated infections. Inspite of these shortcomings, the survey provides background information on infection rate in our facility, particularly from ESBL producing enterobacteriaceae, which may be useful for the hospital IPC intervention programme.

Conclusion:

In conclusion, the prevalence of clinical infections among hospitalised patients in our facility is high but the rate due to ESBL-producing enterobacteriaceae is low. In spite of this, there is need for continuous surveillance of antimicrobial resistance in enterobacteria-ceae and other nosocomial pathogens as part

of the IPC programme, with implementation of control measures to reduce the incidence of infections from ESBL and other multidrug resistant pathogens in our hospital.

Acknowledgments:

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