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Original Research Article

Antibiotic Susceptibility Pattern of Extended Spectrum Betalactamase (ESBL) Producers and other Bacterial Pathogens in Kano, Nigeria

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Abstract

Purpose: *To evaluate the antibiotic susceptibility pattern of various bacterial pathogens including extended spectrum betalactamase (ESBL) producers in Kano, Nigeria.*
Method: *A total of 604 consecutive clinical samples obtained from Aminu Kano Teaching Hospital (AKTH), Kano between January and July 2010 were analyzed for bacterial pathogens using standard microbiological techniques for the isolation and identification of pathogens. Antibiotics susceptibility tests including, ESBL screening and confirmation, were carried out by disc diffusion technique using Clinical Laboratory Standard Institute (CLSI) criteria.*
Results: *Ten different types of bacteria genera were observed from nine different clinical samples. E. coli was the most frequently isolated bacteria (30.5 %) followed by Staphylococcus aureus (21.3 %). ESBL producers showed high-level resistance against the quinolones, aminoglycoside and cotrimoxazole but were sensitive to carbapenems and levofloxaccin. Non-ESBL organisms showed increased resistance to amoxicillin-clavulanate, ceftazidime, cotrimoxazole, tetracycline and amoxycillin. The prevalence of ESBL producers was 12.8 %. E. coli, Klebsiella pneumonia and Pseudomonas aeruginosa showed ESBL production of 17.3, 14.9 and 10.0 %, respectively.*
Conclusion: *The findings of this study suggest that regular surveys should be carried out in this locality to provide baseline data that would always be of clinical relevance in the treatment of patients and to detect the emergence of multiple antibiotic resistance strains.*

Keywords: *Antibiotic susceptibility, Multiple antibiotic resistance, Extended spectrum betalactamase, Bacterial pathogens*

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INTRODUCTION

Before the discovery of penicillin, which initiated the antibiotic era, the prognosis for people with infectious diseases, such as bacterial pneumonia, tuberculosis and staphylococcal infections, was poor [1]. Today antibiotics are widely prescribed for various bacterial infections with a high degree of success. However, the unfortunate outcome of the wide-scale use of antibiotics is the development of antimicrobial resistance, an adaptive response in which microorganisms begin to tolerate an amount of drug to which it was previously susceptible. The development of mechanisms for circumventing or inactivating antibiotic drugs is due largely to the genetic versatility and adaptability of microbial populations [2].

Beta-lactam antimicrobial agents are the most common drugs for the treatment of bacterial infections and account for over 50 % of global antibiotic consumption [3]. Bacterial resistance to B-lactam antibiotics has significantly increased in recent years and has been attributed to the spread of plasmid mediated B-lactamases. Some of these organisms have produced new forms of the older enzymes such as the extended-spectrum B-lactamases (ESBLs) that can hydrolyze newer celphalosporins and aztreonam [4].

Extended-spectrum b-lactamases (ESBLs) represent an important mechanism of resistance in Enterobacteriacaeae, because they inactivate penicillin, narrow- and extended-spectrum cephalosporins, and aztreonam [5]. This study is focused on the prevalence and antibiotic susceptibility pattern of ESBL producers in bacterial isolates of urogenital infections and other bacterial pathogens in Kano, Nigeria.

EXPERIMENTAL

Media

MacConkey agar, Blood agar base, Mueller Hinton agar, Salmonella Shigella agar were all manufactured by Oxoid, Bassingoke, United Kingdom. The antibiotic discs; ofloxacin 15 µg, ceftazidime 30 µg, ceftriazone 30 µg,ciprofloxacin 10 µg, amoxicillin/clavulanate 30 µg, gentamicin 10 µg, levofloxacin 10 µg, amoxycillin 30 µg, erythromycin 15µg, cotrimoxazole 25 µg, and cloxacillin 10 µg (all from Abbot Laboratories, UK) were placed after the plates were confirmed to have dried, and incubated for 18 - 24 h. *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 were used as control.

Samples

A total of 604 clinical samples as listed above obtained from patients at Amino Kano teaching Hospital from various service points between January and July 2010 were analyzed for bacterial pathogens by standard microbiological procedures [6].

The clinical samples consisted of the following: urine (N = 302), wound swab (N = 151), ear swabs (N = 78), catheter tips (N = 34), semen (N = 3), endocervical swabs (N = 12), throat swabs (N = 3) and umbilical cord swab (N = 6).

The samples were inoculated on blood and MacConkey agar. Salmonella Shigella agar was included for enteric pathogens. All were incubated aerobically at 37 ^{o}C for 18 - 24 h. Anaerobic culture was not carried out. Gram stain, motility and biochemical tests were used to identify the various isolates.

Antibiotic susceptibility test

This was carried out by disc diffusion technique, (WHO modified) in accordance with the CLSI criteria and interpreted accordingly [7]. Mueller Hinton culture plates were inoculated by dipping a sterile cotton wool swab into a suspension of the overnight growth of the organism prepared to the density of a Mc Farland no 0.5 opacity standard; excess liquid from the swab was expressed before inoculation by spread plate method.

ESBL producers-screening and confirmation

The isolates were tested against third generation cephalosporins (cefodoxime, cefotaxime and ceftriaxone) using Clinical Laboratory Standard Istitute (CLSI) recommended, WHO modified Kirby Bauer disc diffusion method [8]. Zone diameters were interpreted using the revised National Committee on Clinical Laboratory (NCCL) Standard document [14]. Isolates with reduced susceptibility to cefpodoxime (≤ 17 mm) cefotaxime (≤ 27 mm) and ceftriaxone (≤ 25 mm) were considered to be possible ESBL producers.

Phenotypic confirmation test was carried-out using Double Disc Synergy test. Disc containing the standard 10 ug of cefpodoxime and 30 ug of ceftazidime/ceftriaxone, are placed 15 mm apart (edge to edge); with amoxicillin-clavulanic acid disc containing 10 ug of the latter compound mounted exactly at their center. After 16 - 20 h of incubation at 35 ^{o}C, any enhancement of the zone of inhibition between a beta-lactam disk and that containing the beta-lactamase inhibitor is indicative of the presence of an ESBL [9].

Statistical analysis

The data were computed as mean and standard deviation as well as in percentage. Chi-square analysis was carried out by Epi info Version 6 software. $P < 0.05$ was accepted as statistically significant.

RESULTS

The age group prevalence of infected patients is presented on Table 1. The age group that had the highest number of isolates was 0 - 10 years (27.8 %) while > 70 years (0.9 %) had the least.

The difference was statistically significant X^2 = 356.9, df = 7, $p < 0.001$.

Table 1: Prevalence by age of infected patients

Age (years)	N	Prevalence (%)
0-10	168	27.8
11-20	71	11.8
21-30	166	27.5
31-40	78	12.9
41-50	47	7.7
51-60	35	5.8
61-70	33	5.5
>70	6	0.9

X^2 = 356.9, df = 7, $p < 001$

Table 2 shows the distribution of bacterial pathogens by clinical samples and gram staining reactions. 75 % of the isolates showed gram negative result while 24.8 % were Gram positive cocci. Urine sample constituted the largest number of specimens (50 %) followed by wound swab (25 %). *E. coli* was the most frequently isolated organism (30.5 %) followed by *S. aureus* (21.3 %).

The antibiotic susceptibility data for the bacterial pathogens, which shows high level of resistance against various antibiotics, are presented in Table 3.

Most pathogens were resistant to commonly used antibiotics such as tetracycline, cotrimoxazole and amoxycillin. *E. coli* and *Klebsiella pneumonia* showed 14 and 16 % susceptibility respectively against amoxicillin/clavulanate and 43 and 21 % respectively against ceftazidime. However, ceftriaxone, gentamicin and levofloxacin showed over 60 % susceptibility against most bacterial pathogens.

Table 4 shows the percentage prevalence of extended spectrum beta-lactamase producers among urogenital pathogens.

E. coli had the highest prevalence of 17.3 % followed by *Klebsiella pneumonia* 14.9 % while the least was *Proteus vulgaris* 2.4 %. The difference however, was not statistically significant (X^2 = 9.22, df = 4, p = 0.05).

The antibiotic susceptibility pattern of ESBLs against six antibiotics is presented in Table 5. Besides exhibiting high level resistance against the cephalosporins, these pathogens also show resistance to other groups of antibiotics such as the aminoglycoside, fluoroquinolones and cotrimoxazole.

However, the cabapenemes and levofloxacins showed encouraging results above 60 % (57 - 69 %).

DISCUSSION

The prevalence of resistance to antibiotics varies greatly from one geographical area to another as well as between hospitals within community, mainly because of the differences in antimicrobial usage and infection control practices [10].

The age group prevalence of high infectious rate observed in infants and older children when exposed to bacterial pathogens could be attributed to their low level of immunity, other researchers made similar observations in their reports [11]. The majority of the bacterial isolates 75.2 %, observed in the study were Gram negative bacilli mainly of the Enterobacteriaceae.

Table 2: Distribution of bacterial pathogens by clinical samples and Gram-staining reactions

Isolate	UR	CT	ES	WS	ST	ECS	TS	SM	UBC	Total	%
Gram-negative											
E. coli	136	9	20	15	0	5	0	0	1	186	30.5
K. pneumonia	58	3	10	10	0	6	1	2	0	90	14.8
P. mirabilis	25	3	10	10	0	1	0	0	0	49	9.0
P. vulgaris	39	0	7	2	0	0	1	1	0	50	8.2
P. aeruginosa	24	9	16	10	0	0	1	0	0	60	9.8
Enteropathic E. coli	0	0	0	0	10	0	0	0	0	10	1.6
Salmonella typhi	0	0	0	0	5	0	0	0	0	5	0.8
Enterobacter Spp	2	0	0	0	0	0	0	0	0	2	0.3
M. morgani	1	0	0	0	0	0	0	0	0	1	0.2
S. marcescens	1	0	0	0	0	0	0	0	0	1	0.2
Gram-positive											
E. faecalis	16	0	0	4	0	0	0	0	0	20	3.3
S. aureus	0	10	15	100	0	0	0	0	5	130	21.3
Total	302	34	78	151	15	12	3	3	6	604	

Key: UR = urine, CT = catheter tip, ES = ear swab, WS = wound swab, ST = stool, ECS = endocervical swab, TS = throat swab, SM = semen, UBC = umbilical cord swab

Table 3: Antibiotic susceptibility of bacteria pathogens isolated from clinical samples in AKTH

Isolated Pathogens	Total	Sensitive to isolates N(%)										
		CN	AUG	OFL	CAZ	CRO	CIP	COT	TE	AMX	ERY	CLOX	LEV
E. coli	186	130(69.9)	26(14.0)	110(59.1)	80(43.0)	140(75.3)	124(66.67)	10(5.38)	8 (4.30)	12 (6.45)	NT	NT	160(86.0)
*E. coli**	10	6 (60.0)	4 (40.0)	7 (70.0)	4 (40.0)	7 (70.0)	6 (60.0)	2(20.0)	2(20.0)	4(40.0)	NT	NT	8 (80.0)
K. pneumonia	90	70 (77.8)	15(16.7)	70 (77.8)	40(21.5)	70 (77.8)	60 (66.7)	5 (5.6)	10(11.1)	5 (5.6)	NT	NT	80 (88.9)
P. mirabilis	49	40 (81.6)	20(40.8)	42 (85.7)	20(40.8)	41 (83.7)	30 (61.2)	3 (6.1)	3 (6.1)	5 (10.2)	NT	NT	40 (81.6)
P. vulgaris	50	30 (60.0)	20(40.0)	35 (70.0)	15(30.0)	40 (80.0)	30 (60.0)	2 (4.0)	4 (8.0)	8 (16.0)	NT	NT	40 (80.0)
P. aeruginosa	60	40 (66.7)	0 (0)	40 (66.7)	10(16.7)	40 (66.7)	15 (25.0)	0(0)	0(0)	0(0)	NT	NT	50 (83.3)
Salmonella typhi	5	3 (60.0)	1 (20.0)	3 (60.0)	2 (40.0)	4 (80.0)	3 (60.0)	0(0)	0(0)	1(20.0)	NT	NT	3 (60.0)
Enterobacter Spp	2	1 (50.0)	0 (0)	0 (0)	0(0)	1 (50.0)	1 (50.0)	0(0)	0(0)	0(0)	NT	NT	1 (50.0)
Morganella m	1	0 (0)	0 (0)	0 (0)	0(0)	1 (100)	0(0)	0(0)	0(0)	0(0)	NT	NT	0 (0)
Serratia m	1	0 (0)	0 (0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	0(0)	0(0)	NT	NT	1 (100)
E. faecalis	20	10 (50.0)	10(50.0)	5 (25.0)	0(0)	0 (0)	8 (40.0)	0(0)	0(0)	0(0)	15(75.0)	15(75.0)	15(75.0)
S. aureus	130	70 (53.8)	40(30.8)	80 (61.5)	5 (3.8)	30 (23)	70 (53.8)	0(0)	0(0)	0(0)	80(61.5)	60(45.2)	80(61.5)

Key: *Enteropathogenic *E.coli*, CN = gentamicin, AUG = amoxycillin/clavulanate, OFL = ofloxacin, CAZ = ceftazidime, CRO = ceftriaxone, CIP = ciprofloxacin, COT = cotrimoxazole, TE = tetracycline, AMX = amoxycillin, ERY = erythromycin, CLOX = cloxacillin, LEV = levofloxacin, NT = not tested

This is in agreement with the reports of other researchers [12]. *E. coli* (30.5 %) was the most frequently isolated. It also accounted for most of all urinary isolates giving 45.0 %. This observation is in agreement with the findings of other research workers [13]. The preponderance of this organism may be due to the fact that it constitutes a large proportion of the intestinal flora and with low hygienic practices, it will be expected to be isolated in diseases such as urinary tract infection, wound infections and other bacterial infections.

The prevalence of ESBL producers in this study was 12.8 %, which is higher than an earlier study in Kano [14] that reported 9.25 %, but much lower than a report of 36.2 % from Ebonyi State in Nigeria [15]. However, these results are higher than the report of 2.6 % from researchers in Bosnia and Herzegovina [16], and also 8.9 % from Saudi Arabia [17]. These may be attributed to different cultures of antibiotic usage in states where studies were carried out.

E. coli and *Klebsiella pneumoniae* showed ESBLs prevalence of 17.3 % and 14.9 % respectively in the present study. These values were lower than the reports from India [18]. However, both reports are at variance with another report from Nigeria [19] in which *Klebsiella pneumoniae* had ESBL prevalence of 35.3 % while *E. coli* had 27.3 %. The antibiotic susceptibility of ESBL *E. coli* was 46.1, 7.6, 30.8 and 57.7 % when tested against gentamicin, cotrimoxazole, ciproflaxacin and levofloxacin respectively. Against imipenem and meropenem ESBL producing *E. coli* showed an antibiotic susceptibility of 69.2 % and 61.5 %, respectively. Other researchers [20] also reported encouraging antibiotic susceptibility result with imipenem and meropenem.

The predominating presence of *S. aureus* (76.9 %) on the isolates in wound swab, agrees with earlier reports [21]. It is important to mention that its high level resistance characteristics observed in this study is also in agreement with other reports [22]. *S. aureus* showed antibiotic susceptibility of 30.8, 3.8, 23, 0, 0, 0 and 45.2 % when tested with amoxicillin/clavulanate, ceftazidime, ceftriaxone, cotrimoxazole, tetracyclin, amoxicillin and cloxacillin. *E. coli* was also observed to have shown very poor susceptibility to all the antibiotics mentioned which are all routine and commonly used drugs

Table 4: Prevalence of extended-spectrum betalactamase (ESBL) producers among Gram-negative urogenital pathogens

Bacteria isolate	No. of isolates screened	Positive	Negative	Prevalence (%)
E. coli	150	26	124	17.3
Klebsiella pneumoniae	67	10	57	14.9
Proteus mirabilis	29	2	27	6.8
Proteus vulgaris	41	1	40	2.4
Pseudomonas aeruginosa	33	2	31	6.0
Total	320	41	280	12.8

X^2=9.22, df = 4, p = 0.05

Table 5: Antibiotic susceptibility pattern of ESBL producers

Pathogen	No. of isolates	Sensitive isolates N(%)					
		CN	***COT***	***CIP***	***IMP***	***MEP***	***LEV***
E. coli	26	12(46.1)	2(7.6)	8(30.8)	18(69.2)	16(61.5)	15(57.7)
Klebsiella pneumonia	10	5(50)	0(0)	4(40)	7(70)	6(60)	6(60)
Proteus mirabilis	2	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)
Proteus vulgaris	1	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)
Pseudomonas aeruginosa	2	1(50)	0(0)	0(0)	1(50)	0(0)	1(50)

Key: *CN = gentamicin, COT = cotrimoxazole, CIP = ciprofloxacin, IMP = imipenem, MEP = meropenem, LEV = levofloxacin*

that are completely losing their efficacy against increasing wave of bacterial resistance.

Many clinical laboratories (as well as the wider medical community) are not fully aware of the importance of ESBLs and how to detect them; laboratories may also lack the resources to curb the spread of these resistance mechanisms [23]. This ignorance and possible financial constraints may be responsible for the continuous dissemination of ESBLs worldwide.

Despite the rapidity with which new chemotherapeutic agents are introduced, bacteria have shown a remarkable ability to develop resistance to these agents. In view of the steady rise in bacteria resistance to antibiotics, it has become very important to carry out *in vitro* antimicrobial susceptibility testing before prescription.

CONCLUSION

Regular studies of the antibiotic susceptibility pattern of isolates commonly observed in a locality will guide therapeutic judgment and enhance antibiotic prescribing. Such surveys will yield the first clue when multi drug resistant isolates are encountered to prevent therapeutic failures that could be fatal in the management of patients.

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