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CEPHALOSPORIN RESISTANCE AMONG CLINICAL GRAM NEGATIVE BACTERIAL ISOLATES OBTAINED FROM MURTALA MUHAMMED SPECIALIST HOSPITAL, KANO

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Abstract: A total of 300 Gram negative bacterial isolates from various specimens were obtained from the Microbiology Department of Murtala Muhammed Specialist Hospital, Kano which include; 30 (10%) stool samples, 135 (44.9%) urine samples, 45(15%) sputum and 90 (30%) swabs. Standard biochemical tests were carried out to identify the isolates as *Klebsiella spp*. 145/300, *Escherichia coli* 96/300 (31.9%), *Pseudomonas spp.* 34/300 (11.3%) and *Proteus spp.* 25/300 (8.6%). The isolates were subjected to sensitivity test using Disc Diffusion method. Following antibiotic susceptibility testing, the resistance of the isolates to cefoperazone, cefotaxime, ceftriaxone, ceftazidime and cefepime was 47.5% (143/300), 100% (300/300), 89% (268/300), 99.3% (299/300) and 82.1% (247/300) respectively. This result indicates that the rate of resistance of Gram negative bacteria to third and fourth generation cephalosporin is increasing at an alarming rate which further indicates the need to find measures to limit or control antimicrobial resistance to antibiotics.
Keywords: Cephalosporin, Resistance, Bacterial isolates, Hospital.

INTRODUCTION

Cephalosporins are the most frequently prescribed class of antibiotics that are structurally and pharmacologically related to the penicillins. Like the penicillins, cephalosporins have a beta-lactam ring structure that interferes with synthesis of the bacterial cell wall and so are bactericidal (Beers, 2003).

Resistance to β-lactam antimicrobial agents especially extended-spectrum cephalosporin and other antimicrobial agents among clinical isolates of Gram-negative bacteria is on the rise worldwide (Pfaller *et al.,* 2000). Infections with multi-drug resistant Gram-negative pathogens impose a significant and increasing burden on both patients and healthcare providers. After the widespread adoption of broad-spectrum cephalosporins in the 1980s, *Enterobacteriaceae* producing extended spectrum beta-lactamases have become endemic in hospitals and communities worldwide. Infection with an extended-spectrum beta-lactamase producing Gram-negative pathogen particularly *Escherichia coli*, or *Klebsiella pneumoniae,*

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is associated with greater mortality, an increase in the length of hospital stay and hospitalization costs, and delay in treatment compared with infection due to non-ESBL producing organisms (Braykov *et al.,* 2013).

MATERIALS AND METHODS

Collection and Confirmation of Clinical Isolates

The clinical isolates that were used in this research were identified Gram negative bacterial isolates obtained from the microbiology laboratory of Murtala Muhammed Specialist Hospital, Kano State. Gram's staining and other biochemical tests were carried out according to standard procedures to confirm the identity of the isolates.

Biochemical Tests

Indole Test

The test organisms were inoculated into Bijou bottles containing 3ml of sterile peptone water and incubated at 37^0C for 24hrs. After incubation, 0.5ml of Kovacs reagent was added, shaken gently and examined for a red color in the surface layer within 10mins (Cheesbrough, 2006).

Urease Test

The test organisms were inoculated into about 3ml of urea agar medium in Bijou bottles and incubated at 37^0C for 24hrs. After incubation, the bottles were observed for a pink colour which indicates a positive urease test (Cheesbrough, 2006).

Citrate Utilization Test

Using a sterile Straight loop, a suspension of the test organism in normal saline was streaked first on the slope of the Simmon's citrate agar in Bijou bottles and then stabbed at the bottom of the medium and incubated at 35^0C for 48hrs. A bright blue color indicates a positive citrate test (Cheesbrough, 2006).

Triple Sugar Iron (TSI) Agar Test

Using a sterile straight loop, the butt of the TSI agar in a test tube was stabbed with a suspension of the isolate and the slope was streaked, followed by incubation at 37^0C for 24hrs. Change in the butt to yellow indicates lactose fermentation while a yellow slope indicates glucose fermentation.

Inoculum Standardisation

Using a sterile inoculation loop, a loopful of colony from a 24hour culture of the isolates were dispensed in sterile normal saline to match the 0.5 McFarland's standard for sensitivity tests as described by NCCLS, (1999).

Antibiotic Susceptibility Tests

The clinical Gram negative bacterial isolates were tested for their susceptibility to the cephalosporin antibiotic discs using the modified Kirby-Bauer disc diffusion technique (Cheesbrough, 2006). Standardized inocula of the isolates were swabbed onto the surface of prepared and solidified Mueller-Hinton Agar (MHA) in separate petridishes. This was followed by placing the antibiotic discs separately at regular intervals onto the inoculated MHA plates and then incubated aerobically at 35^0C for 24hrs (Ankur *et al.,* 2008). Zone diameters produced by the isolates were measured in millimeters with a ruler. The isolates were then classified as resistant, intermediate or sensitive based on the interpretative chart updated according to the current NCCLS standards (NCCLS, 2002).

RESULTS

A total of 300 Gram negative bacterial isolates were collected from the sampling site (i.e Murtala Muhammed Specialist Hospital,Kano), with the majority originating from urine samples which had a frequency of 135 (*Klebsiella spp.* 76, *Escherichia coli* 48, *Pseudomonas spp*. 8, *Proteus spp*. 3),followed by swabs with 91 samples (*Klebsiella spp*. 44, *Escherichia coli* 8, *Pseudomonas spp*.23, *Proteus spp*. 16), then sputum with 45 samples (*Klebsiella spp.* 20, *Escherichia coli* 15, *Pseudomonas spp.* 3, *Proteus spp*. 7) and lastly stool which had the least number with 30 samples only (*Klebsiella spp*. 5, *Escherichia coli* 25, *Pseudomonas spp.* 0, *Proteus spp*. 0) (Table 1). Following Gram's staining and biochemical characterization, the isolates were identified as *Klebsiella spp*., *Escherichia coli, Pseudomonas spp*., and *Proteus spp*. *Klebsiella spp*. had the highest frequency of 145 (48.2%) , followed by *Escherichia coli* with a frequency of 96 (31.9%), then *Pseudomonas spp*. 34 (11.3%) and lastly *Proteus spp*. with a frequency of 26 (8.6%) (Table 2).

Of the five different cephalosporin antibiotic discs used for susceptibility testing, cefoperazone (CFP,Oxoid 30µg) showed the highest activity against the Gram negative bacterial isolates used. Ninety one (30.2%) of the isolates were highly susceptible to cefoperazone, 67 (22.2%) intermediately susceptible and 143 (47.5%) were resistant. Followed by cefepime (FEP,Oxoid 30µg) with 49 (16.3%) susceptible isolates, 5 (1.7%) intermediately susceptible isolates, and 247 (82.1%) resistant isolates. Ceftriaxone (CRO,Oxoid 30µg) also showed some activity against the Gram negative bacterial isolates with 10 (3.3%) highly susceptible isolates, 23 (7.6%) intermediately susceptible isolates and 268 (89%) resistant isolates. The highest resistance was seen in cefotaxime (CTX ,Oxoid

30µg) and ceftazidime (CAZ ,Oxoid 30µg) where the isolates showed a 100% and 99.3% resistance respectively (Table 3).

TABLE 1: Distribution of Bacterial Isolates Based on Samples

Bacterial Isolate	Sputum	Urine	Swabs	Stool	TOTAL
Klebsiella spp.	20	76	44	5	145
Escherichia coli	15	48	8	25	96
Pseudomonas spp	3	8	23	0	34
Proteus spp	6	3	16	0	25
TOTAL	45(15%)	135(44.9%)	91(30.2%)	30(10%)	**300**

Table 2: Overall Occurrence of Different Gram negative Bacterial Species

Bacterial isolate	Frequency	Percentage (%)
Klebsiella spp.	145	48.2
Escherichia coli	96	31.9
Pseudomonas spp.	34	11.3
Proteus spp.	25	8.6
TOTAL	**300**	**100**

Table 3: Susceptibilty of the different Gram negative bacterial isolates

Bacterial isolate	CFP			CAZ			CRO			CTX			FEP		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Klebsiella spp.	44	27	74	0	0	145	3	15	127	0	0	145	21	3	121
Escherichia coli	29	24	43	0	1	95	3	7	86	0	0	96	22	1	73
Pseudomonas spp.	12	7	15	0	1	33	0	1	33	0	0	34	4	0	30
Proteus spp.	6	9	11	0	0	26	4	0	22	0	0	26	2	1	23
TOTAL	**91**	**67**	**143**	**0**	**2**	**299**	**10**	**23**	**268**	**0**	**0**	**301**	**49**	**5**	**247**

CFP = Cefoperazone , CAZ = Ceftazidime, CRO = Ceftriaxone, CTX = Cefotaxime, FEP = Cefepime, S = Susceptible, I = Intermediate, R = resistant

DISCUSSION

Of the total 300 Gram negative bacteria isolated from various clinical specimens, majority were from urine 44.9% (135/300), followed by swabs 30.2% (91/300), sputum 15% (45/300), and lastly stool with 10% (30/300) (Table 1). Among the bacterial isolates, *Klebsiella* spp. was the most predominant with 48.2%, followed by *Escherichia coli* 31.9%, *Pseudomonas* spp. 11.3% and *Proteus* spp. 8.6% (Table 2). This is in contrast with the research carried out by Garba and Yusha'u (2012) where *E.coli* was the most predominant isolate with 32.7%. The high occurrence of members of the *Enterobacteriaceae* among the isolates may be due to poor hygienic practices among the patients which were reported as important factors leading to the spread of ESBLs due to non- ESBLs producing strains acquiring plasmids responsible for ESBLs production (Denman and Burton, 1992).

Among the antibiotics, cefoperazone showed the most activity against *Klebsiella* spp. with a susceptibility percentage of 30.3% which relates with the research carried out by Zaki (2007), where the percentage activity of cefoperazone against *Klebsiella* spp. was 43.3%. In contrast however, cefotaxime, ceftazidime and ceftriaxone activity against *Klebsiella* spp. in this research was found to be 0%,0% and 3% respectively whereas in the research by Zaki (2007), it was found to be 43.3%, 35.3% and 42% respectively. The high occurrence of cephalosporin resistance in *Klebsiella* spp. observed in this research is of great concern since infections caused by the bacterium (particularly respiratory tract infections) are very common in this part of Nigeria due to the contagious nature and resistance of the organism to harsh conditions, which may be due to the presence of capsules that gives some level of protection to the cells (Paterson and Bonomo, 2005). All the other Gram negative bacterial isolates were also relatively susceptible to cefoperazone, with *Escherichia coli* showing 30.2% susceptibility, *Pseudomonas* spp. with 35.3%, and *Proteus* spp. with 23.1%.

The Gram negative isolates were highly resistant to cefotaxime, ceftriaxone and ceftazidime. *E.coli* exhibited 99%, 89.6% and 100% resistance to cefotaxime,ceftriaxone and ceftazidime respectively. The high resistance of *E. coli* to cephalosporins appears to be a warning sign that agents of this class need to be used with greater caution and more wisdom, especially in urinary tract or intra-abdominal infections. The percentage resistance to these antibiotics was also high in *Klebsiella* spp., *Pseudomonas* spp. and *Proteus* spp. (Table 3). This result is in agreement with the results of the study carried out by Sasirekha and Shivakumar (2012), where the rate of resistance of Gram negative bacterial isolates to ceftazidime, cefotaxime and ceftriaxone was 85.7%, 85.7% and 71.42% respectively. It is also in line with the results

obtained by Ashour and El-Shariff (2009) where *E.coli* exhibited 66.2% and 55.7% resistance to cefotaxime and ceftazidime respectively. The highest rates of resistance may result from the common use of cephalosporins in treatment which will lead to increasing antimicrobial resistance rates that can be controlled by limiting the use of such antibiotics as reported by Meyer *et al.,* (1997).

About 82.1% of the isolates showed high resistance to cefepime *Klebsiella* spp., *E.coli, Pseudomonas* spp. and *Proteus* spp. showing 83.4%, 76%, 88.2% and 88.5% respectively. This result is in contrast with the results obtained by Sharif et al., (2014) where the resistance of *E.coli, Klebsiella, Pseudomonas* and *Proteus* spp. to cefepime was 65.1%,32.2%, 80% and 0% respectively. These results indicate that using antibiotics such as cefepime should be controlled and there should be restricted policies for usage of these antibiotics in order to control antibiotic resistance (Sharif *et al.,* 2014).

CONCLUSION

In conclusion, resistance to third and fourth generation cephalosporins was high (47.5, 100, 89, 99.3 and 82.1% for cefoperazone, ceftazidime, ceftriaxone, ceftazidime and cefepime respectively) among the clinical bacterial isolates used in this research.

RECOMMENDATIONS

Having observed the high rate of resistance of Gram negative bacteria to the selected third and fourth generation cephalosporin antibiotics used in this research, which is probably due to the indiscriminate prescription and use, immediate "checkup" on antibiotics prescribed for infections caused by these organisms will be of great impact in reducing the rate of resistance within the immediate locality. It is therefore important to curb these problems of antibiotic overuse through rational use of antimicrobial agents and constant antimicrobial sensitivity surveillance should be encouraged to help clinicians provide safe and effective empiric therapies. This will serve as an important means of reducing the selective pressure that will help reduce emergence and re-emergence of resistant organisms and also ensure quick recovery of patients in the hospitals.

Also, knowledge of the local distribution of pathogens, their susceptibility patterns and prompt initiation of effective antimicrobial treatment are essential in patients suffering from multidrug-resistant infections caused by GNB through alertness, awareness campaigns and improvement in healthcare services, sanitation and hygienic character in all sectors of life.

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