

## Resistance to Third-Generation Cephalosporins and Other Antibiotics by Enterobacteriaceae in Western Nigeria

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**Abstract: Problem statement:** The emergence and spread of resistance to third-generation cephalosporins are threatening to create species resistant to all currently available agents. The most common cause of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamases and many of the 2nd and 3rd-generation penicillins and cephalosporins were specifically designed to resist the hydrolytic action of major  $\beta$ -lactamases. However new  $\beta$ -lactamases emerged against each of the new classes of  $\beta$ -lactams that were introduced and caused resistance. This study was designed to determine the rate of resistance to 3rd-generation cephalosporins and other classes of antibiotics by the Enterobacteriaceae in this environment. **Approach:** One hundred bacteria isolates belonging to the family Enterobacteriaceae identified from different clinical specimens between October and December 2007 using standard bacteriological methods. These were subjected to antibiotic susceptibility testing to third-generation cephalosporins and other classes of antibiotics which included quinolones and an aminoglycoside using the Kirby-Bauer method of disc diffusion test. **Results:** Out of the total number of Enterobacteriaceae isolated in the study period, only 54.8% of the klebsiella species isolated were sensitive to ceftazidime, 48.4% to ceftriaxone and 30.7% to cefotaxime. With *Escherichia coli* however, the susceptibility pattern to the 3rd-generation cephalosporins was better (65.6% were sensitive to ceftazidime, 62.5% to ceftriaxone and 71.9% to cefotaxime). In proteus species, the susceptibility pattern was generally poor to the three classes of antibiotics (50% were sensitive to ceftazidime and ceftriaxone, 0% to cefotaxime, 33.3% to ciprofloxacin, 50% to gentamycin and 0% to amoxicillin/clavulanate). **Conclusion/Recommendations:** The poor susceptibility to amoxicillin/clavulanate demonstrated by all the isolates in this study showed the probability of new beta-lactamases production. Further studies therefore need to be done in this environment to determine the types of the  $\beta$ -lactamases produced by the Enterobacteriaceae here, the prevalent rate of such isolates and the molecular analysis of the new  $\beta$ -lactamases produced.

**Key words:** Enterobacteriaceae, third-generation cephalosporins, resistance

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### INTRODUCTION

Increasing resistance to 3rd-generation cephalosporins has become a cause for concern especially among Enterobacteriaceae that cause nosocomial infections<sup>[1]</sup>. This emergence and spread of resistance are also threatening to create species resistant to all currently available agents. Approximately 20% of *Klebsiella pneumoniae* infections and 31% of Enterobacter species infections in intensive care unit in the United States now involve strains not susceptible to 3rd-generation cephalosporins<sup>[2]</sup>

Among the wide array of antibiotics,  $\beta$ -lactams are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use<sup>[3]</sup>. The most

common cause of bacterial resistance to  $\beta$ -lactam antibiotics is the production of  $\beta$ -lactamases. Many of the 2nd and 3rd generation penicillins and cephalosporins were specifically designed to resist the hydrolytic action of major  $\beta$ -lactamases. However, new  $\beta$ -lactamases emerged against each of the new classes of  $\beta$ -lactams that were introduced and caused resistance. In fact, since  $\beta$ -lactam antibiotics came into clinical use,  $\beta$ -lactamases have co-evolved with them<sup>[4]</sup>.

The latest in the arsenal of these enzymes has been the evolution of the Extended Spectrum  $\beta$ -Lactamases (ESBLs). These enzymes are commonly produced by many members of Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae* and efficiently hydrolyze oxyimino-cephalosporins

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conferring resistance to third-generation cephalosporins such as cefotaxime, ceftazidime and ceftriaxone and to monobactams such as aztreonam<sup>[5]</sup>. Their activity is however inhibited by clavulanic acid *in vitro*<sup>[6]</sup>

Plasmid-mediated Extended Spectrum  $\beta$ -Lactamases (ESBLs) were first identified in a *Klebsiella pneumoniae* isolate in Germany in 1983<sup>[7]</sup>.

Since then, the infections caused by ESBL-producing members of the family Enterobacteriaceae have rapidly increased<sup>[8]</sup>. Organisms producing these beta-lactamases may also be resistant to quinolones and aminoglycosides by different mechanisms<sup>[9]</sup>. Another worrying development has been the detection of plasmid-mediated carbapenemases, which can inactivate antibiotics such as imipenem and meropenem, which may necessitate avoidance of 3<sup>rd</sup>-generation cephalosporins in the management of serious infections caused by the enterobacteriaceae<sup>[9]</sup>.

Since its isolation in 1983 in Germany, ESBLs have spread rapidly to Europe, United States and Asia and are now found all over the world<sup>[10]</sup>. Being plasmid-mediated, they are easily transmitted among members of Enterobacteriaceae thus facilitating the dissemination of resistance not only to  $\beta$ -lactams but to other commonly used antibiotics such as quinolones and aminoglycosides<sup>[9]</sup>.

Despite world-wide use of  $\beta$ -lactam antibiotics, the distribution of the enzymes responsible for resistance to oxyimino-cephalosporins and carbapenems is far from being uniform. Some hospitals in the United States seem to have no or low ESBLs, whereas in other hospitals, as many as 40% of *K. pneumoniae* isolates have been reported to be ceftazidime resistant as a result of ESBLs production<sup>[11]</sup>.

This study was therefore conducted to determine the rate of resistance to 3<sup>rd</sup>-generation cephalosporins by Enterobacteriaceae in this environment. The resistance pattern to other classes of antibiotics such as the quinolones, aminoglycosides and amoxicillin/clavulanate will also be determined.

## MATERIALS AND METHODS

This study was carried out in the diagnostic Medical Microbiology Laboratory of University College Hospital, Ibadan, Nigeria.

One hundred bacteria isolates belonging to the family Enterobacteriaceae were identified from different clinical specimens between October and December, 2007 using standard bacteriological methods. These clinical specimens included sputum, urine, wound swabs and biopsies, ear swabs, tracheal aspirate, conjunctival swabs, blood and throat swabs.

Information regarding the name, sex, age, ward or clinic, type of specimen taken and clinical diagnosis made were also obtained.

The antibiotic susceptibility patterns of these isolates to 3<sup>rd</sup>-generation cephalosporins and other antibiotics were determined using the Kirby-Bauer method of disc diffusion test as described by Qin et al in 2004<sup>[12]</sup>.

The isolates were tested against the following antibiotics; ceftazidime (30 ug), cefotaxime (30 ug), ceftriaxone (30 ug), cefuroxime (30 ug), gentamycin (10 ug), ciprofloxacin (5 ug), sparfloxacin (5 ug), Pefloxacin (5 ug) and amoxicillin/clavulanate (30 ug).

Control organism used as standard was *Escherichia coli* NCTC 10418.

## RESULTS

One hundred bacterial pathogens belonging to the family Enterobacteriaceae were isolated and identified from various clinical specimens. These included sputum 8 (8%), Urine 30 (30%), wound swabs and biopsies 35 (35%), ear swabs 12 (12%), tracheal aspirate 5 (5%), blood 4 (4%), conjunctival swabs 1 (1%) and throat swabs 5 (5%) (Table 1).

These clinical specimens were obtained from patients who consisted of 42 (42%) males and 58 (58%) females. The age range of these patients was between 7 days and 77 years.

62 (62%) of the isolates were *Klebsiella* species, 32 (32%) *Escherichia coli* and 6 (6%) *Proteus* species (Table 2).

*In vitro* activities of nine different antibiotics against the bacterial isolates are illustrated in Table 3. These included 3<sup>rd</sup>-generation cephalosporins, quinolones and an aminoglycoside.

Table 1: Distribution of isolates in clinical specimens

Clinical specimens	Klebsiella	<i>E. coli</i>	Proteus	Total (Among
	species	species	species	all isolates)
	No. (%)	No. (%)	No. (%)	No. (%)
Sputum	6(9.7)	2(6.3)	0(0.0)	8(8.0)
Ear Swab	8(12.9)	2(6.3)	2(33.3)	12(12.0)
Conjunctival Swabs	1(1.6)	0(0.0)	0(0.0)	1(1.0)
Urine	12(19.4)	18(56.3)	0(0.0)	30(30.0)
Blood	4(6.5)	0(0.0)	0(0.0)	4(4.0)
Wound swabs	21(33.9)	10(31.1)	4(66.7)	35(35.0)
Tracheal aspirate	5(8.0)	0(0.0)	0(0.0)	5(5.0)
Throat Swabs	5(8.0)	0(0.0)	0(0.0)	5(5.0)
Among all specimens	62(100.2)	32(100.2)	6(100.0)	100(100.0)

Table 2: Distribution of bacterial pathogens

Organism	No.	(%)
<i>Klebsiella</i> species	62	62
<i>Escherichia coli</i>	32	32
<i>Proteus</i> species	6	6
Total	100	100

Table 3: Antibiotic susceptibility pattern of isolates

Isolates	Antimicrobial agents No. (%)								
	Ceftazidime	Ceftriaxone	Cefotaxime	Cefuroxime	Gentamycin	Ciprofloxacin	Sparfloxacin	Pefloxacin	Amoxicillin/ clavulanate
Klebsiella species (62)	34(54.8)	30(48.4)	19(30.7)	24(38.7)	30(48.4)	36(58.1)	39(62.9)	37(59.7)	24(38.7)
<i>E. coli</i> (32)	21(65.6)	20(62.5)	23(71.9)	19(59.4)	16(50.0)	21(65.6)	18(56.3)	25(62.5)	12(37.5)
<i>Proteus</i> species (6)	3(50.0)	3(50.0)	3(50.0)	0(0.0)	2(33.3)	3(50.0)	2(33.3)	3(50.0)	0(0.0)

Taking into account the total number of Enterobacteriaceae isolates in the study period only 34 (54.8%) of the *Klebsiella* species isolated were sensitive to Ceftazidime, 30 (48.4%) to Ceftriaxone, 19 (30.7%) to Cefotaxime which were all 3<sup>rd</sup>-generation cephalosporins. There was poor susceptibility to gentamycin 30 (48.4%) but susceptibility to quinolones was a little better than in the 3<sup>rd</sup>-generation cephalosporins as demonstrated in ciprofloxacin 36 (58.1%) sparfloxacin 39 (62.9%) and pefloxacin 37 (59.7%).

With *Escherichia coli*, the susceptibilities were higher to the 3<sup>rd</sup>-generation cephalosporins (Table 3) and the quinolones but intermediate in gentamycin. The susceptibility pattern in *Proteus* species was generally low to the three classes of antibiotics.

All the Enterobacteriaceae isolates demonstrated very poor susceptibility patterns to amoxicillin/clavulanate especially *Proteus* species which showed nil susceptibility to it (Table 3). This may be an indication that there is production of the new  $\beta$ -lactamases in these isolates.

## DISCUSSION

Extended spectrum beta-lactamase producing organisms vary in their susceptibility to different oxyimino-beta-lactams and ,despite resistance to some they may appear sensitive to others<sup>[8]</sup>.

The present study has shown that there is probability of production of new  $\beta$ -lactamases by the Enterobacteriaceae isolated in this environment. This is demonstrated by the very poor susceptibility to amoxicillin/clavulanate by all the isolates.

There was an increased resistance of *Klebsiella* species isolates to cefotaxime as compared to ceftazidime. 69.3% of *Klebsiella* isolates were resistant to Cefotaxime as compared to 45.2% resistance to Ceftazidime. This is in keeping with the findings of Kumar *et al.*<sup>[1]</sup> where 85% of *K. pneumoniae* were resistant to Cefotaxime but only 37% to Ceftazidime<sup>[1]</sup>. However, contrary to the results in the same study mentioned above, resistance to cefotaxime was lower in *Escherichia coli* than to Ceftazidime.

In a study conducted by Spanu *et al.*<sup>[13]</sup>, gentamycin and tobramycin typically demonstrated

poor *in vitro* activity against ESBL-producing organisms<sup>[13]</sup>. The same was the case in the present study as gentamycin, the only aminoglycoside used in the study demonstrated poor *in vitro* activities against *Klebsiella* species, *E. coli* and *Proteus* species. Such resistant isolates pose serious problems to the physicians as therapeutic options are limited. ESBLs are plasmid-mediated and multidrug resistance is a characteristic feature of strains producing ESBLs. Our study confirms this observation in *Klebsiella* and *Proteus* species, as these isolates were resistant to different classes of antibiotics (3<sup>rd</sup>-generation cephalosporins, quinolones, aminoglycosides and amoxicillin/clavulanate).

For infections caused by ESBL-producing *E. coli* or *Klebsiella* species, treatment with imipenem or meropenem has been associated with the best outcomes in terms of survival and bacteriological clearance<sup>[14]</sup>. Cefepime and piperacillim-tazobactam have been less successful, ceftriaxone cefotaxime and ceftazidime have failed even more often, despite the organisms susceptibility to the antibiotic *in vitro*<sup>[15]</sup>. Some patients have responded to aminoglycoside or quinolone therapy, but in a recent comparison of ciprofloxacin and imipenem for bacteremia involving ESBL-producing *K. pneumoniae*, imipenem produced the better outcome<sup>[16]</sup>.

## CONCLUSION

The correlation between *in vitro* resistance and treatment failure is imperfect, but resistance undoubtedly increases mortality, morbidity and costs in many settings. This has led to a plethora of governmental and agency reports advocating less antibacterial use, better antibacterial use, better infection control and the development of new antibacterials.

The evidence that better prescribing can reduce resistance rates is mixed and although changes to hospital regimens may reduce one resistance problem, other opportunistic bacteria may fill the vacant niche.

There are no  $\beta$ -lactams in development that can treat infections with organisms producing some of the new  $\beta$ -lactamases. Available agents therefore need to be used judiciously and infection control measures

implemented in outbreak situations to prevent the further spread of pathogens with these all-too-successful mechanisms of resistance.

Further studies need to be done to determine the types of the new  $\beta$ -lactamases produced by the enterobacteriaceae isolates in this environment, the prevalent rate of such isolates and the molecular analysis of the new  $\beta$ -lactamases produced.

#### ACKNOWLEDGMENT

The researchers appreciate Mrs A. Agboola and Mrs O. Olola for their technical contribution as well as Mrs Popoola for her secretarial assistance.

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