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

A.O. Okesola and O. Makanjuola
Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, University College Hospital, Ibadan, Nigeria, West Africa

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 amoxycillin/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

INTRODUCTION

Increasing resistance to 3rd-generation cephalosporins has become a cause for concern especially among Enterobacteriaceae that cause nosocomial infections[1]. 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 cephalosporin[2].

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[3]. 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[4].

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.
conferring resistance to third-generation cephalosporins such as cefotaxime, ceftazidime and ceftriaxone and to monobactams such as aztreonam\cite{5}. Their activity is however inhibited by clavulanic acid in vitro\cite{6}.

Plasmid-mediated Extended Spectrum β-Lactamases (ESBLs) were first identified in a *Klebsiella pneumoniae* isolate in Germany in 1983\cite{7}.

Since then, the infections caused by ESBL-producing members of the family Enterobacteriaceae have rapidly increased\cite{8}. Organisms producing these beta-lactamases may also be resistant to quinolones and aminoglycosides by different mechanisms\cite{9}. 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 3rd-generation cephalosporins in the management of serious infections caused by the enterobacteriaceae\cite{10}.

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\cite{10}. Being plasmid-mediated, they are easily transmitted among members of Enterobacteriaceae thus facilitating the dissemination of resistance not only to β-lactams but to other commonly used antibiotics such as quinolones and aminoglycosides\cite{10}.

Despite world-wide use of β-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\cite{11}.

This study was therefore conducted to determine the rate of resistance to 3rd-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 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 3rd-generation cephalosporins, quinolones and an aminoglycoside.

### 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 3rd-generation cephalosporins, quinolones and an aminoglycoside.

### MATERIALS AND METHODS

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

One hundred bacterial pathogens belonging to the family Enterobacteriaceae were isolated and identified from various clinical specimens. 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 3rd-generation cephalosporins and other antibiotics were determined using the Kirby-Bauer method of disc diffusion test as described by Qin et al in 2004\cite{12}.

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 3rd-generation cephalosporins, quinolones and an aminoglycoside.

### MATERIALS AND METHODS

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

One hundred bacterial pathogens belonging to the family Enterobacteriaceae were isolated and identified from various clinical specimens. 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 3rd-generation cephalosporins and other antibiotics were determined using the Kirby-Bauer method of disc diffusion test as described by Qin et al in 2004\cite{12}.

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 3rd-generation cephalosporins, quinolones and an aminoglycoside.

| Clinical specimens | Klebsiella species No. (%) | E. coli No. (%) | Proteus species No. (%) | Total (Among all isolates) No. (%) |
|-------------------|---------------------------|----------------|------------------------|-----------------------------------|
| Sputum            | 6(9.7)                    | 2(3.3)         | 0(0.0)                 | 8(8.0)                            |
| Ear Swab          | 8(12.9)                   | 2(3.3)         | 0(0.0)                 | 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(35.9)                  | 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)                        |

| Organism          | No. (%) |
|-------------------|---------|
| Klebsiella species| 62      |
| *Escherichia coli*| 32      |
| Proteus species   | 6       |
| Total             | 100     |
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 3rd-generation cephalosporins. There was poor susceptibility to gentamycin 30 (48.4%) but susceptibility to quinolones was a little better than in the 3rd-generation cephalosporins as demonstrated in ciprofloxacin 36 (58.1%) and pefloxacin 37 (59.7%).

With *Escherichia coli*, the susceptibilities were higher to the 3rd-generation cephalosporins (Table 3) than 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 β-lactams in these isolates.

**DISCUSSION**

Extended spectrum beta-lactamase producing organisms vary in their susceptibility to different oximino-beta-lactams and despite resistance to some they may appear sensitive to others[8].

The present study has shown that there is probability of production of new β-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.*[1] where 85% of *K. pneumoniae* were resistant to Cefotaxime but only 37% to Ceftazidime[1]. 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.*[13], gentamycin and tobramycin typically demonstrated poor *in vitro* activity against ESBL-producing organisms[13]. 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 (3rd-generation cephalosporins, quinolones, aminoglycosides and amoxycillin/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[14]. Cefepine and piperacillin-tazobactam have been less successful, ceftriaxone cefotaxime and ceftazidime have failed even more often, despite the organisms susceptibility to the antibiotic *in vitro*[15]. 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[16].

**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 β-lactams in development that can treat infections with organisms producing some of the new β-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 β-lactamases produced by the enterobacteriaceae isolates in this environment, the prevalent rate of such isolates and the molecular analysis of the new β-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.

REFERENCES

1. Kumar, M.S., V. Lakshmi and R. Rajagopalan, 2006. Occurrence of extended-spectrum beta-lactamases among Enterobacteriacea spp. isolated at a tertiary care institute. Ind. J. Med. Micro., 24: 208-211. http://www.bioline.org.br/request?mb06060

2. Paterson, D., 2006. Resistance in gram-negative bacteria: Enterobacteriacea. Am. J. Inform. Control, 34: 520-528. http://linkinghub.elsevier.com/retrieve/pii/S0196655306008534

3. Bronson, J.J. and J.F. Barrett, 2001. Quinolone, eveninomycin, glycycline, carbenem, lipopeptide and Cephem. Antibacterials in clinical development. Curr. Med. Chem., 8: 1775-1793. DOI: 10.2174/0929867013371653

4. Medeiros, A.A., 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin. Infect. Dis., 24: S19-S45. http://www.ncbi.nlm.nih.gov/pubmed/8994778

5. Jacoby, G.A. and A.A. Medeiros, 1991. More extended-spectrum β-lactamases. Antimicrob Agents Chemother., 35: 1697-1704. http://aac.asm.org/cgi/reprint/35/9/1697

6. Girlich, D., L. Poireland and A. Leelaporn et al., 2001. Molecular epidemiology of the integron-located VEB-1 extended-spectrum beta-lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. J. Clin. Microbiol., 39: 175-182. DOI: 10.1128/JCM.39.1.175-182.2001

7. Knothe, H., P. Shah, V. Kremery, M. Antal and S. Mitsuhashi, 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection, 11: 315-317. DOI: 10.1007/BF01641355

8. George, A., M.D. Jacoby and Luisa Silvia Munoz-Price, 2005. The new β-lactamases. N. Engl. J. Med., 352: 380-391. http://content.nejm.org/cgi/content/full/352/4/380

9. Bradford, P.A., 2001. Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology and detection of this important resistance threat. Clin. Microbiol. Rev., 14: 933-951. DOI: 10.1128/CMR.14.4.933-951.2001

10. Kliebe, C., B.A. Nies, J.F. Meyer, R.M. Tołxdorff-Neutzling and B. Weidemann, 1985. Evolution of plasmid coded resistance to broad spectrum cephalosporins. Antimicrob. Agents Chemotherapy., 28: 302-307. http://aac.asm.org/cgi/content/abstract/28/2/302

11. Burwen, D.R., S.N. Banerjee and R.P. Gaynes, 1994. Ceftazidime resistance among selected nosocomial gram-negative bacilli in the United States. J. Infect. Dis., 170: 1622-1625. http://cat.inist.fr/?aModele=afficheN&cpsidt=3336301

12. Qin, D., S.J. Weissman and M.F. Chestnut et al., 2004. Kirby-bauer disc approximation to detect inducible third-generation cephalosporin resistance in Enterobacteriaciæ. Ann. Clin. Microbiol. Antimicrobl., 3:13-13. DOI: 10.1186/1476-0711-3-13

13. Spanu, T., F. Luzzanno, M. Perilli, Q. Amicosante, A. Toniolo and G. Fadda, 2002. Italian ESBLs study group. Occurrence of Extended spectrum β-lactamases in members of family enterobacteriaciæ in Italy. Implication of resistance to β-lactams and other antimicrobial agents. Antimicrob. Agents Chemother., 46: 196-202. DOI: 10.1128/AAC.46.1.196-202.2002

14. Burgess, D.S. and R.G. Hall, 2004. In vitro killing of parenteral beta-lactams against standard and high inocula of extended-spectrum beta-lactamase and non-esbl producing Klebsiella pneumoniae: Treatment outcome of patients receiving imipenem or ciprofloxacin. Clin. Infect. Dis., 38: 243-251. DOI: 10.1086/380645