ORIGINAL PAPER

Nig. J. Pharm. Res. 2015, 1, pp. 40-45
ISSN 0189-8434

Multidrug Resistant and Extended-Spectrum Beta-Lactamase (ESBL) Producing *Proteus Mirabilis* from Tertiary Hospitals in Four States in Southwest Nigeria

*O. S. Alabi.[1ABCDEF], O. E. Adeleke [1CDEF], O. A. Adegoke[2B], O. F. Gbadeyan [3B] and O. Ejilude [4B]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.
[2]Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria.
[3]Department of Microbiology and Parasitology, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria.
[4]Department of Medical Microbiology and Parasitology, Sacred Heart Hospital, Lantoro, Abeokuta, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Multidrug resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing Gram-negative bacteria pose great threat to antibiotic treatment of life threatening infections worldwide.
Objectives: This study investigated the occurrence and distribution of MDR and ESBL producing *Proteus mirabilis* among clinical isolates collected from tertiary hospitals in four states in Southwest Nigeria.
Materials and Methods: One hundred and eight (108) none-duplicated *P. mirabilis* collected from microbiology units of tertiary hospitals in four states in Southwest Nigeria namely; Oyo, Osun, Ogun and Lagos state, after authentication with standard bacteriological method, were subjected to antibiotic susceptibility test against ten selected antibiotics using disc-diffusion method. Presumptive production of ESBL was determined by double disc synergy test among isolates with MDR phenotype that showed resistance to any of the third generation cephalosporin antibiotics.
Results: Of the 108 clinical isolates of *P. mirabilis* collected from Oyo (39.8%), Osun (25.9%), Ogun (21.3%) and Lagos (13%) states, 60 (55.6%) showed MDR phenotype. Among the 60 MDR isolates collected in Oyo (50%), Lagos (10%), Ogun (21.7%) and Osun (18.3%), 66.7%, 66.7%, 30.8% and 9.1% of the isolates produced ESBL, respectively.
Conclusion: This study recorded the occurrence of ESBL and MDR *P. mirabilis* in all the four states but higher percentage of ESBL-production among MDR *P. mirabilis* in two of the states, Oyo and Lagos. Hence, there is need for adequate monitoring of antibiotic use to prevent increased rate of ESBL-positive MDR *P. mirabilis* in these states and others in the nearest future.
Keywords: Extended-spectrum beta-lactamase, Multidrug resistant *Proteus mirabilis*, phenotypic screening, Tertiary hospital

INTRODUCTION

Proteus mirabilis is among the commonly implicated opportunistic pathogens causing infections in different anatomical sites of the body in both hospital and community settings (O'Hara *et al*., 2000). It is recognized as a frequent nosocomial pathogen and has been implicated in several nosocomial outbreaks of infections (Chikere *et al*., 2008; Chukwu *et al*., 2011; Nwanze *et al*., 2007; Samuel *et al*., 2010). The bacteria have been ranked third to *E. coli* as the cause of uncomplicated cystitis and pyelonephritis particularly in hospital-acquired cases involving long term catheterization (Coker *et al*., 2000). It causes alkalinization of urine through its unique urease enzymes produced in the infected kidney of patients with anatomical abnormalities which may then lead to struvite stone formation and thus kidney failure if not addressed (Chauhan *et al*., 2008). It also cause burn wound infections, bactereaemia, prostatitis in men and nosocomial pneumonia (Coker *et al*., 2000; Mehta *et al*., 2007). *P. mirabilis* is intrinsically resistance to nitrofurantoin and tetracycline but naturally susceptible to beta-lactams,

aminoglycosides and fluoroquinolones (O'Hara *et al*., 2000). However, over the years, resistance to these agents including the extended-spectrum beta-lactams and other classes of antibiotics such as macrolides, monobactams and in rear cases carbapenems have been reported in this organism (Kyabaggu *et al*., 2007; Okesola *et al*., 2009). Resistance to extended-spectrum beta-lactams in most Gram-negative bacteria have been credited to the presence of extended-spectrum beta-lactamases (ESBLs) which are usually encoded on genes present on transferable plasmids within the bacteria concern (Aladag and Durak, 2009; AlZarouni *et al*., 2008). The ESBLs which are mutants of the classical beta-lactamases, was first reported in Germany in 1983 from *Klebsiella pneumoniae* (Knothe *et al*., 1983) and since then have been reported in *Serratia marcenscens*, *Escherichia coli*, *Pseudomonas aeruginosa* and other Gram-negative bacilli in several parts of the world (Kiratisin *et al*., 2008; Okesola and Fowotade, 2012). *P. mirabilis* like *Klebsiella pneumoniae* and *Serratia marcenscens* have been reported to produce extended-spectrum beta-lactamases at increased rates that cannot be eradicated by third generation cephalosporins (Francesco *et al*., 2001) and the ESBL-positive strains frequently exhibits co-resistance to aminoglycosides, fluoroquinolones and other classes of antibiotics (De-Champs *et al*., 2000; Luzzaro *et al*., 2000; Winokur *et al*., 2001). In Nigeria, several studies have demonstrated the presence of ESBL-producers among Gram-negative bacilli and the prevalence have been reported to vary between geographical areas depending on the pattern of usage of beta-lactams, particularly extended-spectrum beta-lactam antibiotics (Babypadmini and Appalaraju, 2004; Winokur *et al*., 2001). Apart from the report of high prevalence (70%) of ESBL-producers among clinical isolates of *Proteus* species in Ibadan, Southwest Nigeria by Okesola in 2010 (Okesola and Adeniji, 2010), and the report of 27.3% ESBL prevalence in *P. mirabilis* among other Gram-negatives by Ogbolu *et al* (2011), there have been no other report focusing on the occurrence of ESBL-producing MDR *P. mirabilis* in southwest Nigeria.

This study therefore, was carried out with the view to determine the occurrence and distribution of ESBL producing clinical isolates of MDR *P. mirabilis* in each of the four states in Southwest Nigeria and to evaluate statistically the level of the differences in the antibiotic resistance of both ESBL and Non-ESBL producing MDR *P. mirabilis* isolates from the states.

Materials and Methods

Collection and authentication of clinical isolates

One hundred and eight (108) none-duplicated clinical isolates of P. mirabilis were collected from the microbiology units of tertiary hospitals in four states of southwest Nigeria namely: Oyo, Osun, Ogun and Lagos state. They were authenticated in the laboratory using Microbact identification kit (Oxoid). The isolates were recorded in the laboratory to be from ten different clinical specimens namely; urine, wound swabs, ear swabs, high vagina swabs, eye swabs, endo-cervical swabs, pus, sputum, peritoneal effluents and stool.

Antibiotic Susceptibility testing

The pure culture of the clinical isolates were subjected to antibiotic susceptibility testing against ten antibiotics namely, 10µg gentamicin, 30µg amikacin, 5µg ciprofloxacin, 30µg cefoxitin, 30µg ceftazidime, 30µg cefotaxime, 10µg amoxicillin, 20/10µg amoxicillin-clavulanic acid, 30µg aztreonam and 10µg imipenem, using disc-diffusion test. The 0.5McFarland standard dilutions of the isolates were inoculated on the surface of Mueller Hinton agar by surface spreading after drying the surface in a sterile incubator at 35^{o}C. The zones of growth inhibition were interpreted according to the CLSI 2011 guidelines (CLSI, 2011). Clinical isolates of *P. mirabilis* that demonstrated resistance to two or more classes of antibiotics were regarded as MDR isolates (Magiorakos *et al*., 2012).

Presumptive ESBL screening

All MDR isolates showing resistance to any of the third generation cephalosporins such as cefotaxime and ceftazidime, were selected for phenotypic detection of ESBL production.

Phenotypic detection of ESBL production

Phenotypic detection of ESBL production was assessed by the double disk synergy test with 30µg ceftazidime and 30µg cefotaxime discs placed 20mm around 20/10µg amoxicillin-clavulanic acid disc on the surface of Mueller Hinton agar plate inoculated with the MDR isolate suspension diluted to 0.5 McFarland standard and spread evenly over the agar surface to give a single layered cell with sterile swabs.

RESULTS

Out of the 108 clinical isolates of *P. mirabilis* collected in this study, 43.5%, 22.2%, 21.3% were from wound swabs, ear swabs and urine specimen (Table 1). Others include eye swabs (1.9%), high vagina swabs (0.9%), endo-cervical swabs (2.8%), peritoneal effluent (1.9%), sputum (1.9%), stool (2.8%) and pus (0.9%). The highest percentage of the isolates from wound swabs, 85.7%, 47.8% and 42.9%, were recorded from Lagos, Ogun and Osun state respectively while the highest percentage of the isolates from urine (32.6%) was recorded in Oyo state as shown in Table 1. The overall percentage of ESBL-producers among the MDR isolates was 48.3% while MDR isolates from Oyo, Lagos, Ogun and Osun recorded 66.7%, 66.7%, 30.8% and 9.1% respectively (Table 2). Among the MDR isolates from urine, wound swab and ear swabs, 57.1%, 44.4% and 41.7% produced ESBL respectively as well as all the MDR isolates from Endo-cervical and eye swabs (Table 3).

The antibiotic susceptibility profile of the ESBL-producing MDR *P. mirabilis* revealed a high resistance against cefotaxime (100%), ceftazidime (≥50%), amoxicillin (100%), gentamicin (≥50%) and ciprofloxacin (≥50%) across the four states under investigation (Table 4).

DISCUSSION

Most of the isolates were found to be from wound, urine and ear swabs in each of the four states (Table 1), this corresponded with the findings in previous studies on clinical isolates of *Proteus* species in Nigeria and other countries. Okesola (2010) reported higher isolation of *Proteus* species from urine (32%) followed by wound (28%) and then ear swabs (18%) out of 50 isolates from six different clinical sources in 2009 from western Nigeria (Okesola and Adeniji, 2010). Similar report was presented by Ogbolu *et al*., (2011) where most of the *P. mirabilis* isolated alongside the other Gram-negative bacilli from 2005 to 2007 was from wound, urine and ear swabs. This confirmed *P. mirabilis* as one of the commonest causative agents of wound, ear and urinary tract infections.

Table 1: Number and Percentage distribution of *Proteus mirabilis* in clinical specimens according to each state

S/N	Clinical specimens	Total (108)	STATES			
			Oyo (43)	Osun (28)	Ogun (23)	Lagos (14)
1	Urine	23 (21.3%)	14 (32.6%)	4 (14.3%)	5 (21.7%)	0 (0%)
2	Wound swab	47 (43.5%)	12 (27.9%)	12 (42.9%)	11 (47.8%)	12 (85.7%)
3	Ear swab	24 (22.2%)	8 (18.6%)	7 (25%)	7 (30.4%)	2 (14.3%)
4	High vagina swab	1 (0.9%	1 (2.3%)	0 (0%)	0 (0%)	0 (0%)
5	Eye swab	2 (1.9%)	2 (4.7%)	0 (0%)	0 (0%)	0 (0%)
6	Endo-cervical swab	3 (2.8%)	3 (7%)	0 (0%)	0 (0%)	0 (0%)
7	Peritoneal effluent	2 (1.9%)	2 (4.7%)	0 (0%)	0 (0%)	0 (0%)
8	Sputum	2 (1.9%)	0 (0%)	2 (7.1%)	0 (0%)	0 (0%)
9	Stool	3 (2.8%)	0 (0%)	3 (10.7%)	0 (0%)	0 (0%)
10	Pus	1 (0.9%)	1 (2.3%)	0 (0%)	0 (0%)	0 (0%)

Table 2: Percentage ESBL production among MDR clinical isolates of *P. mirabilis* in each state

States	Number of *P. mirabilis* isolates collected	Number of *P. mirabilis* found to be MDR	Number of MDR *P. mirabilis* found to produce ESBL	% ESBL producing MDR *P. mirabilis*
Oyo	43	30	20	66.7
Osun	28	11	1	9.1
Ogun	23	13	4	30.8
Lagos	14	6	4	66.7
Total	108	60	29	48.3

Its ability to survive on instruments and catheters for long period and ability to form biofilms are some of the characteristics that qualified the organism as potential opportunistic pathogen among long term catheter users and wound infections mostly among in-patients (Al-Duliami *et al*., 2011).

The percentages of ESBL producing MDR *P. mirabilis* in each state was found to vary with Oyo and Lagos state having the highest (66.7%) compared to that of other states. However, the overall percentage of ESBL producing MDR *P. mirabilis* was 48.3% in this study. Okesola and Adeniji reported 70% prevalence of ESBL production among clinical isolates of *Proteus* species in Ibadan, western Nigeria (Okesola and Adeniji, 2010). This is much higher when compared with the overall percentage of ESBL producing MDR *P. mirabilis* for the four states in this study. However, comparing Okesola's value with that of Oyo state alone, confirms the high prevalence of ESBL producing *P. mirabilis* in Ibadan, Oyo state as reported by Okesola and Adeniji (2010).

The antibiotic susceptibility profile of the ESBL positive MDR isolates to the selected antibiotics used in this study was observed to vary among the states. ESBL-positive MDR isolates recorded high resistance against the third generation cephalosporins as well as against ciprofloxacin and gentamicin in all the states under investigation. This confirms the report by some scientist that ESBL-positive strains frequently exhibits co-resistance to some antibiotics order than extended-spectrum beta-lactams (De-Champs *et al*., 2000; Luzzaro *et al*., 2000; Winokur *et al*., 2001). The result of 50% resistance rate obtained by Okesola and Makanjuola (2009) on the susceptibility of *Proteus* species to third generation cephalosporins when compared with the result in this study (100%

resistance) suggest that the resistance rate of *Proteus* species to cephalosporins, especially third generations, is on the increase in Southwest, Nigeria as being reported in other countries of the world (Francesco *et al*., 2001). Based on the susceptibility test carried out in this study, the use of amikacin and particularly carbapenems in the

Table 3: Percentage ESBL producing P. mirabilis among collected MDR clinical isolates in relation to each clinical specimen

Clinical samples	Number of *P. mirabilis* isolates collected	Number of *P. mirabilis* found to be MDR	Number of MDR *P. mirabilis* found to produce ESBL	% ESBL producing MDR *P. mirabilis*
Wound swab	47	27	12	44.4
Urine	23	14	8	57.1
Ear swab	24	12	5	41.7
Eye swab	2	1	1	100
High vagina swab	1	1	NP	NP
Peritoneal effluent	2	2	1	50
Pus	1	1	NP	NP
Stool	3	NP	NP	NP
Endo-cervical swabs	3	2	2	100
Sputum	2	NP	NP	NP
Cumulatively	108	60	29	48.3

Key: NP = Not Present

Table 4: Percentage Antibiotic Resistance Pattern of ESBL and None ESBL Producing Proteus mirabilis in each state

Antibiotics	Oyo state (n = 30)		Osun state (n = 11)		Ogun state (n = 13)		Lagos state (n = 6)	
	ESBL +ve (n = 20)	ESBL -ve (n = 10)	ESBL +ve (n = 1)	ESBL -ve (n = 10)	ESBL +ve (n = 4)	ESBL -ve (n = 9)	ESBL +ve (n = 4)	ESBL -ve (n = 2)
GN	25%	80%	100%	60%	50%	22.2%	50%	100%
AK	20%	0%	0%	10%	0%	0%	0%	0%
CIP	10%	0%	100%	30%	50%	33.3%	50%	100%
FOX	10%	0%	0%	20%	0%	22.2%	0%	0%
CAZ	90%	10%	0%	20%	50%	11.1%	50%	0%
CTX	100%	10%	100%	60%	100%	22.2%	75%	0%
ATM	25%	0%	0%	20%	25%	22.2%	0%	0%
IPM	0%	0%	0%	0%	25%	11.1%	0%	0%
AML	100%	90%	100%	90%	100%	100%	100%	50%
AMC	20%	20%	0%	30%	25%	55.6%	25%	0%

Key: GN=Gentamicin, AK=Amikacin, CIP=Ciprofloxacin, AML=Amoxicillin, AMC=Amoxicillin/clavulanic acid, CAZ=Ceftazidime, CTX=Cefotaxime, FOX=Cefoxitin, ATM=Aztreonam, IPM=Imipenem

treatment of ESBL-positive MDR *P. mirabilis* infections will provide effective therapeutic outcome in the four states.

CONCLUSION

The percentage of ESBL-producing MDR *P. mirabilis* varies among the four states, however rational use of antibiotics particularly the extended-spectrum beta-lactams and good infection control programmes must be encouraged in our hospitals in all the four states so as to avert cases of outbreaks of ESBL-producing MDR bacteria like *P. mirabilis* born out of selection pressure due to irrational use of extended-Spectrum antibiotics. The use of narrow-spectrum antibiotics after culture and sensitivity test rather than the use of extended-spectrum antibiotics for empirical treatment of infections may go a long way to prevent high level of ESBL-producing bacteria outbreak in Southwest Nigeria.

REFERENCES

Aladag, M. O. and Durak, Y. (2009). Investigating Some Antibiotics, Plasmids Profile and ESBL Characteristic of *Klebsiella pneumonia* Isolated from Urinary System Infection. *World Appl Sci* J. 6: 630-636.

Al-Duliami, A. A., Nauman, N. G., Hassan, A. S. H. and Al-Azawi, Z. H. (2011). Virulence Factors of *Proteus mirabilis* isolated from Patients Otitis Media in Baquba and It's Peripheries. *Diyala Journal of Medicine*. 1: 69 – 75.

Al-Zarouni, M., Senok, A., Rashid, F., Al-Jesmi, S. M., Panigrahi, D. M. (2008). Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. *Medical Principles and Practices*. 17: 32-36.

Babypadmini, S. and Appalaraju, B. (2004). Extended-spectrum beta-lactamase in urinary isolates of *E.coli* and *Klebsiella pneumoniae* – prevalence and susceptibility pattern in a tertiary care hospital. *Indian J. Med. Microbiol*. 22: 172–174.

Chauhan, C. K., Joseph, K. C., Parekh, B. B. and Joshi, M. J. (2008). Growth and Characterization of Struvite Crystals. *Indian Journal of Pure and Applied Physics*. 46: 507-512.

Chikere, C. B., Omoni, V. T. and Chikere, B. O. (2008). Distribution of Potential Nosocomial Pathogens in an Hospital Environment. *African Journal of Biotechnology*. 7: 3535-3539.

Chukwu, B. F., Okafor, H. U. and Ikefuna, A. N. (2011). Asyptomatic bacteriuria in children with sickle cell anemia at the University of Nigeria teaching hospital, Enugu, South East, Nigeria. *Italian Journal of Pediatrics*. 37: 45.

Clinical and Laboratory Standards Institute (2011). Antimicrobial Susceptibility testing Standards Table 2A *Enterobacteriaceae* M02-A10. 31: 42-46.

Coker, C., Poore, C. A., Li, X., and Mobley, H. L. (2000). Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes and Infection / Institut Pasteur*. 2: 1497-1505.

De-Champs, C., Bonnet, R., Sirot, D., Chanal, C., Sirot, J. (2000). Clinical relevance of *Proteus mirabilis* in hospital patients: a 2-year survey. *J. Antimicrob Chemother*. 45: 537–539.

Francesco, L. A., Mariagrazia, P. B., Gianfranco, A. B., Gianluigi, L. A., Renzo, B. A., Alessanro, Z. C., Ciro, B. C. and Antonio, T. A. (2001). Properties of multidrug-resistant, ESBL-producing *Proteus mirabilis* isolates and possible role of beta-lactam: beta-lactamase inhibitor combinations. *International Journal of Antimicrobial Agents*. 17: 131–135.

Kiratisin, P., Apisarnthanarak, A., Laesripa, C. and Saifon, P. (2008). Molecular Characterization Epidemiology of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates Causing Health Care-Associated Infection in Thailand, Where the CTX-M Family is Endemic. *Antimicrob. Agents Chemother*. 52: 2818-2824.

Knothe, H., Shah, P., Kremery, V., Antal, M. and Mitsuhashi, S. (1983). Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens. Clinical Infection Dis*. 11: 315–317.

Kyabaggu, D., Ejobi, F. and Olela, D. (2007). The Sensitivities to first-line antibiotic therapy of the common urinary tract bacterial infections detected in urine samples at a hospital in metropolitan Kampala (Uganda). *African Health Sciences*. 7: 214-222.

Luzzaro, F., Perilli, M., Amicosante, G., Lombardi, G., Belloni, R., Zollo, A., Bianchi, C. and Toniolo, A. (2000). Properties of multidrug resistance, ESBL producing *P. mirabilis* isolates and possible role of β-lactam/β-lactamase inhibitor combinations. *Int. J. Antimicrob. Agents*. 17: 131-135.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., L. B. Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos,

A., Weber, J. T. and Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 18: 268–281.

Mehta, M., Dutta, P. and Gupta, V. (2007). Bacterial isolates from burn wound infections and their antibiograms: A eight-year study. *Indian J. Plast. Surg*. 40: 25-28.

Nwanze, P. I., Nwaru, L. M., Oranusi, S., Dimkpa, U., Okwu, M. U., Babatunde, B. B., Anake, T. A., Jatto, W. and Asagwara, C. E. (2007). Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern. *Scientific Research and Essay*. 2: 112-116.

O'Hara, C. M., Brenner, F. W. and Andmiller, J. M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*", *Clin. Microbiol. Rev.* 13: 534-546.

Ogbolu, D. O., Daini, O. A., Ogunledun, A., Alli, A. O., Webber, M. A. (2011). High levels of multidrug resistance in clinical isolates of Gram-negative pathogens from Nigeria. *International Journal of Antimicrobial Agents*. 37: 62-66.

Okesola, A. O. and Adeniji, T. W. (2010). Pattern of Extended-Spectrum Beta-Lactamase Production Among Clinical Isolates of *Proteus* Species in Western Nigeria. *World Journal of Medical Sciences*. 5: 94-97.

Okesola, A. O. and Fowotade, A. (2012). Extended-spectrum beta-lactamase production among clinical isolates of *Escherichia coli*. *Int. Res. J. Microbiol*. 3: 140-143.

Okesola, A. O. and Makanjuola, O. (2009). Resistance to Third-Generation Cephalosporins and Other Antibiotics by Enterobacteriaceae in Western Nigeria. *American Journal of Infectious Diseases*. 5: 17-20.

Samuel, S. O., Kayode, O. O., Musa, O. I., Nwigwe, G. C., Aboderin, A. O., Salami, T. A. T., Taiwo, S. S. (2010). Nosocomial Infections and the Challenges of Control in Developing Countries. *Afr. J. Cln. Exper. Microbiol.* 11: 102-110.

Winokur, P. L., Canton, R., Casellas, J. M., and Legakis, N. (2001). Variations in the prevalence of strains expressing an extend-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific Region. *Clin. Infect. Dis.* 32: S94-S103.

Address for correspondence:

Alabi Olumuyiwa Samuel
Department of Pharmaceutical Microbiology, Faculty of Pharmacy,
University of Ibadan, Ibadan, Oyo State, Nigeria.
E-mail: mailpharmmuyiwa@yahoo.com
Telephone: +2348034740434.

Conflict of Interest: None declared
Received: 18 May, 2015
Accepted: 3 July, 2015