World Journal of Medical Sciences 5 (4): 94-97, 2010
ISSN 1817-3055

Pattern of Extended Spectrum Beta-Lactamase Production Among Clinical Isolates of Proteus Species in Western Nigeria

A.O. Okesola and T.W. Adeniji

Department of Medical Microbiology and Parasitology, College of Medicine,
University of Ibadan, University College Hospital, Ibadan, Nigeria, West Africa

Abstract: Proteus species have been reported by some workers as infrequent producers of extended-spectrum beta-lactamases(ESBLS). Moreover, prevalenceand phenotypic characteristics of ESBL may vary between geographical areas. This study was therefore conducted to determine the frequency of production of ESBLS among clinical isolates of Proteus in our institution. A total of 50 isolates of Proteus species were recovered from various clinical specimens which included urine, wound swabs, ear swabs, sputum and high vaginal swabs. ESBL production among these clinical isolates was assessed by the double-disc synergy test. Out of the 50 strains of Proteus species analyzed, 35(70%) were positive for the phenotypic presumptive test while 15(30%) were negative. Highest incidence of ESBL production was found among isolates from urine specimens (37.1%) while the lowest was from wound aspirates (5.7%). The relative susceptibility of Proteus species to cefuroxime, ceftazidime and ceftriaxone were 54%, 22% and 24%, respectively. This study has demonstrated a rather high prevalence of ESBL production among clinical isolates of Proteus species in our environment. This may be due to large scale, indiscriminate use and abuse of these drugs in this environment.

Key words: ESBL · Prevalence · Proteus species · Nigeria

INTRODUCTION

Among the wide array of antibiotics, β-lactams are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use[1]. However, major clinical crisis have resulted from the emergence of resistance to these agents in the past two decades[2]. Moreover, resistance to these β-lactam antimicrobial agents, especially extended-spectrum cephalosporins and other antimicrobial agents is on the rise world wide among clinical isolates of Gram-negative bacteria[3]. The resistance of Gram-negative bacteria to agents such as extended-spectrum cephalosporins, monobactams, carbapenems and β-lactam -B-lactamase inhibitor combinations have emerged through the production of a variety of β-lactamases, alterations in the penicillin binding proteins (PBP), outer membrane permeability and combinations of multiple mechanisms of resistance. Increase in resistance to these agents has paralleled the introduction, administration and over use of β-lactam drugs[4].

Microbial resistance through ESBL production was first reported in the early 1980s in Europe and subsequently in the United States. Soon after the introduction of third-generation cephalosporins in clinical practice, various reports have described world wide outbreaks of infection with ESBL-producing Enterobacteriaceae. The plasmid-encoded derivative of ESBL enable horizontal transmission, a fact, which should result in strict infection control measures[5].

Extended-spectrumβ-lactamases are widespread all over the world, but the prevalence and phenotypic characteristics among clinical isolates may vary between geographical areas[6]. Failure to detect ESBL-mediated resistance has led to treatment failure and contributed to uncontrolled spread of ESBL-producing organisms[7]. Screening for ESBL is therefore needed to sort out patients with these infections in order to perform medically effective treatments and cost-effective isolation[8]. Prevalence of ESBL-producing bacteria in most hospitals remains unknown in spite of numerous reports of nosocomial outbreaks of infection due to these

Corresponding Author: A.O. Okesola, Department of Medical Microbiology and Parasitology, College of Medicine,University of Ibadan, University College Hospital, PMB 5116, Ibadan, Nigeria, West Africa, Tel: 234-803-305-0593.

organisms. Important ESBL-producing Gram-negative bacilli include *Klebsiella pneumoniae*, *Escherichia coli, Proteus mirabilis,* Enterobacter species, *Citrobader freundii*, *Pseudomonas aeruginosa*, Acinetobacter and Stenotrophomonas maltophila[9].

Proteus species have been reported by some workers to be infrequent producers of ESBLS[10,11],however, in a study conducted by Ali *et al*[12], frequency of ESBL production in *Proteus mirabilis* was 61% and in *Proteus vulgaris*, 50%.

This study was therefore conducted to determine the prevalence of ESBL producers among clinical isolates of Proteus species in our environment.

MATERIALS AND METHODS

This study was conducted in the medical microbiology laboratory of University College Hospital, Ibadan, Nigeria, between July and September 2009.

Fifty consecutive, non-duplicated isolates of Proteus species, identified from clinical specimens during the study period by standard bacteriological methods, were included in this study. These clinical specimens included wound aspirates, urine, ear swabs, high vaginal swabs and sputum.

Detection of Extended-spectrum Beta-lactamase Production Using Double - Disc Synergy Test: This test was performed as a disc diffusion test as recommended by CLSI (formerly NCCLS) [13]. Sterile swabs were dipped unto standard bacteria suspensions (0.5 Macfarland turbidity) and these were spread on Mueller Hinton sensitivity agar plates. Amoxycillin-clavulanate (20mg amoxicillin+10mg clavulanate) disc was placed at the centre of the plate. Ceftriaxone (30µg), cefuroxime (30µg) and ceftazidime (30µg) discs were then placed 20mm (centre to centre) from the amoxycillin-clavulanate disc and incubated at 37°C overnight. Enhancement of the zones of inhibition of the cephalosporin β-lactam antibiotic disc (i.e, ceftriaxone, cefuroxime and ceftazidime) caused by the synergy with clavulanate in the amoxycillin/clavulanate disc was taken as an evidence of ESBL production [14].

RESULTS

Fifty isolates of Proteus species were included in this study. A breakdown of the frequency of isolation of this organism from various clinical specimens is illustrated in Table 1.

Table 1: Distribution of Proteus species in the various clinical specimens.

Clinical specimens	No of Proteus species	Percentage (%)
Wound swabs	14	28
Urine	16	32
Ear swabs	9	18
Sputum	1	2
High vaginal swabs	7	14
Wound aspirates	3	6
Total	50	100

Tables 2: Rate of production of ESBLs among clinical isolates of Proteus species

Isolates	Number	Percentage (%)
Positive	35	70
Negative	15	30
Total	50	100

Table 3: Rate of production of ESBLs in Proteus species isolated from various clinical specimens.

Clinical specimens	Number	Percentage (%)
Wound swabs	10	28.6
Ear swabs	5	14.3
Wound aspirates	2	5.7
Urine	13	37.1
High vaginal swabs	5	14.3
Sputum	0	0
Total	35	100

Prevalence of ESBL Production in the Proteus Species: Out of the 50 Proteus species isolates analyzed, 35 (70%) were positive for the phenotypic presumptive test and are therefore ESBL producers, while 15(30%) were negative (Table 2). Breakdown of the rate of production of ESBL in Proteus species identified from the various clinical specimens demonstrated the highest rate in urine specimens 13 (37.1%), followed by wound swabs 10 (28.6%), ear swabs 5(14.3%), high vaginal swabs 5(14.3%) and wound aspirates 2(5.7%) (Table 3).

Relative susceptibility of Proteus species to ceftazidime, ceftriaxone and cefuroxime were 22%, 24% and 54% respectively.

DISCUSSION

This study has revealed that 70% of the 50 Proteus species identified from clinical specimens in our institution were ESBL producers, which indicates a high prevalence in this environment. This result is contrary to that of another study conducted between August 2004

and April 2005 by Khan *et al* which recorded 27.8% detection by double-disc synergy test, but 44% in modified double-disc synergy test which is a more sensitive method [11].

The result of our study did not also support the claim that Proteus species are infrequent producers of ESBL as reported by another study by Shashikala *et al* 2007 and which was carried out between July and December 2004 where 19.4% of Proteus species were recorded to be ESBL producers [10].

The double-disc synergy test employed in this study is a phenotypic presumptive test, which does not detect all ESBL. Some Proteus species may carry the ESBL genes and yet not express them phenotypically,giving rise to a false negative test. These β-lactamases include AmpC and inhibitor resistant TEMS (IRTS). Hyper production of TEM or SHV β-lactamases in organisms with ESBL also may cause false-negative phenotypic confirmatory test results.

Detection of organisms with multiple β-lactamases that may interfere with phenotypic confirmatory test can be accomplished using isoelectric focusing and DNA sequencing [15]. Therefore,molecular techniques will be more reliable in the detection of ESBLS.

CONCLUSION

The results of this study has shown a high prevalence of ESBLs production among Proteus species in our environment. This will not be unconnected with the large-scale, indiscriminate use and abuse of this group of drugs in this environment.

Moreover, since the phenotypic test employed in this study is just a presumptive test and may not be totally reliable, further studies need to be carried out using other more sensitive methods like modified double-synergy test and molecular methods to detect and to determine the prevailing types of β-lactamases in our environment.

Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate use of these drugs is curtailed. Laboratories can also employ the simple method described by Jalier *et al.* [14] routinely to detect ESBL production. Clinicians should also consider ESBL production as a possibility when there is treatment failure with β-lactam antibiotics.

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