

FREQUENCY OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING GRAM NEGATIVE BACILLI AMONG CLINICAL ISOLATES AT CLINICAL LABORATORIES OF ARMY MEDICAL COLLEGE, RAWALPINDI

Ali AM, Rafi S, Qureshi AH

Microbiology Department, Army Medical College Rawalpindi, Pakistan

Background: This study was carried out in Microbiology department of Army Medical College, Rawalpindi to find out the frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates recovered from clinical specimens received from Military Hospital, Rawalpindi. **Methods:** This study was carried out from 1st Jan 2002 to 30th Dec 2002. A total of 812 consecutive Gram-negative bacilli were recovered during the study period from various samples including urine, blood, pus, sputum, high vaginal swab (HVS), aspirates, i/v canula/ Central venous lines (CVP), chest tubes and catheter tips. Extended spectrum beta lactamase detection in these isolates was carried out by Kirby Bauer disc diffusion method on Mueller Hinton agar. A susceptibility disk containing amoxicillin-clavulanate was placed as the inhibitor of beta lactamase in the center of the plate, and cefotaxime, ceftazidime, ceftriaxone and aztreonam disks were placed 30 mm (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino- β -lactam caused by the synergy of the clavulanate in the amoxicillin-clavulanate disk was considered as evidence of ESBL production. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains. **Results:** The frequency of ESBL producing gram negative bacilli among the clinical isolates was 45%.

Keywords: ESBL. Antibiotic. Nosocomial. Enterobacteriaceae

INTRODUCTION

β -Lactam antibiotics are among the most frequently prescribed antimicrobial agents worldwide. The emergence of resistance to these agents in the past two decades has resulted in a major clinical crisis.^{1,2} Gram negative bacteria resistant to agents such as extended-spectrum cephalosporins, monobactams, carbapenems and β -lactam- β -lactamase inhibitor combinations have emerged through the production of a variety of β -lactamases, alterations in the penicillin-binding proteins, outer membrane permeability and combinations of multiple mechanisms of resistance. This increase has paralleled the introduction, administration and overuse of β -lactam drugs.³

Resistance to β -lactam antimicrobial agents, especially extended-spectrum cephalo-sporins and other antimicrobial agents among clinical isolates of gram negative bacteria is on the rise worldwide.^{4,5} These antimicrobial resistant pathogens include extended-spectrum cephalosporin resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens* and *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Recent studies in Taiwan have demonstrated a high prevalence of these antimicrobial-resistant bacteria and a trend of increasing resistance under continued antibiotic selective pressure.⁶

The objective of this study was to determine the frequency of extended-spectrum β -lactamase (ESBL) producing gram-negative bacilli recovered from clinical specimens in our set up.

MATERIAL AND METHODS

A total of 812 consecutive non duplicate gram-negative bacilli recovered from clinical specimens during the study period from 1st Jan 2002 to 30th Dec 2002 were included in the study. These were isolated from various samples including urine, blood, pus aspirate, sputum, chest tube, HVS, i/v canula/ CVP lines and catheter tips received from patients admitted in Military Hospital, Rawalpindi. The samples received were initially inoculated on Blood agar and MacConkey's agar besides Chocolate agar (in case of sputum) where appropriate. Urine samples were cultured on Cystiene lactose electrolyte deficient agar (CLED). The samples were incubated at 37°C under aerobic conditions for

24 hours. The organisms were primarily identified by standard techniques. Confirmation to the species level was done by API 20 E & API 20 NE where required. ESBL production was detected by placing a susceptibility disk containing amoxicillin-clavulanate (20/10 ug) as the inhibitor of beta lactamase in the center of the plate and cefotaxime (30ug), ceftazidime (30ug), ceftriaxone (30ug) and aztreonam (30ug) disks at 30 mm (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino- β -lactam caused by the synergy with clavulanate in the amoxicillin-clavulanate disk was considered as evidence of ESBL production.⁷ *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as control strains. The results were tabulated as frequencies.

RESULTS

Three hundred and sixty six isolates were ESBL producers making a frequency of 45 %. *Enterobacter cloacae* was the most frequent ESBL producer. *Escherichia coli* (45%) was the most frequent organism isolated followed by *Klebsiella pneumoniae* (21%), *Pseudomonas aeruginosa* (19.2%), *Enterobacter cloacae* (4.6%) and *Acinetobacter baumannii* (4.4%) (Table-1).

DISCUSSION

Extended spectrum beta lactamase (ESBL) refers to beta lactamase enzymes produced by gram negative organisms that confer resistance against broad-spectrum beta-lactam antibiotics, normally having activity against gram-negative bacilli. Examples of such antibiotics are cefotaxime, ceftriaxone, ceftazidime and aztreonam.³

The first hospital outbreak of an ESBL producing gram-negative organism was reported in Germany in 1983.⁸ Within one year, nosocomial outbreaks caused by a multidrug resistant *Klebsiella* clone carrying a TEM-3 gene were described in France.⁹ Over the past decade, ESBL-producing Enterobacteriaceae have emerged as serious nosocomial pathogens throughout Europe.¹⁰ Outbreaks have occurred among the most critically ill patients in intensive care units (ICUs).¹¹

The prevalence of ESBL-producing bacteria in most hospitals remains unknown inspite of numerous reports of nosocomial outbreaks of infection due to these organisms. Important ESBL producing gram-negative bacilli include *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis*, enterobacter species, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Stenotrophomonas maltophilia*.¹²

The percentage of isolates expressing ESBL production is variable although a recent study from the United States reported 83 ESBL-producing isolates from 906 consecutive isolates of Enterobacteriaceae over a 20 week period. *Klebsiella pneumoniae* and *Escherichia coli* were the most frequently associated with ESBL production in this study.¹²

During a five-year surveillance study in northern France, the overall proportion of ESBL producers was 11.4% in the 6121 strains of *Klebsiella* species and 47.7% in the 2353 strains of *Enterobacter aerogenes*.¹³

In a national surveillance program conducted in 1996 in Argentina, resistance to extended-spectrum cephalosporins was shown in 48%, 26%, and 8% of *K. pneumoniae*, *Proteus mirabilis*, and *Escherichia coli* isolates respectively.¹⁴

In a study carried out in All India Institute of Medical Sciences, New Delhi during March to June 2001 out of the 678 isolates tested 68% were ESBL producers, which are more than that found in our study. ESBL production was most common in *Klebsiella* spp. (80%).¹⁵

In our study *Enterobacter cloacae* (79%) was the most frequent ESBL producer followed by *Acinetobacter baumannii* (72%) and *Klebsiella oxytoca* (66.66%).

Table-1: Frequency of gram negative bacilli and ESBLs among the recovered isolates (n=812)

Organism	No. of isolates	% of organism	ESBL producers	% of ESBL Producers
<i>E. coli</i>	363	45%	142	39.1%
<i>K. pneumoniae</i>	171	21%	97	57%
<i>K. oxytoca</i>	6	0.7%	4	66.66%
<i>Citrobacter freundii</i>	11	1.35%	4	36.36%
<i>Enterobacter cloacae</i>	38	4.6%	30	79%
<i>Acinetobacter baumannii</i>	36	4.4%	26	72%
<i>Acinetobacter lwofii</i>	8	0.98%	2	25%
<i>Proteus mirabilis</i>	18	2.2%	11	61%
<i>Proteus vulgaris</i>	2	0.2%	1	50%
<i>Aeromonas hydrophila</i>	2	0.2%	1	50%
<i>Providencia rettgeri</i>	1	0.2%	0	0%
<i>Pseudomonas aeruginosa</i>	156	19.2%	47	36.36%
TOTAL	812	100%	366	45%

However these bacteria were infrequently recovered. The relative frequency of ESBL producing Gram negative bacilli in our study is similar to frequency reported from Islamabad, Pakistan¹⁶ with *Escherichia coli* (48%) being the most prevalent organism reported as was found in our study.

The frequency of ESBL producing Gram negative bacilli (35 %) in nosocomial isolates reported from Armed Forces Institute of Pathology, Rawalpindi is lower than our study. *Klebsiella* spp. was the commonest ESBL producing organism reported followed by *Enterobacter cloacae* and *Escherichia coli* whereas *Enterobacter cloacae* was the most frequent ESBL producer in our study.¹⁷

The frequency of ESBL producing gram negative bacilli clinical isolates in our study was 45 %. *Enterobacter cloacae* (79%) was the most frequent ESBL producing organism detected followed by *Acinetobacter baumannii* (72%) and *Proteus mirabilis* (61%) though *Escherichia coli* (45%) was commonest organism identified followed by *Klebsiella pneumoniae* (21%) and *Pseudomonas aeruginosa* beside other gram negative rods

CONCLUSION

The resistance to beta lactam antimicrobial agents among gram-negative bacilli is on the increase in our setup. Laboratories can detect ESBL production by simple technique of Jarlier et al. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless the inappropriate use of these drugs is curtailed. Clinicians should consider ESBL production as a possibility in case of treatment failure with β -lactam antimicrobials.

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Address for Correspondence:

Dr. Arif Maqsood Ali, House No. B- 889, Satellite Town, Rawalpindi, Ph :+92-51-4413383,4429514,4842055

Email: drarifmaqsood@hotmail.com