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Original Article

PREVALENCE OF EXTENDED-SPECTRUM ß-LACTAMASES IN NOSOCOMIAL AND OUTPATIENTS (AMBULATORY)

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ABSTRACT:

Objective: To determine the prevalence of extended-spectrum ß-lactamases (ESBLs) among the bacteria of family Enterobacteriaceae isolated from Nosocomial and outpatients, with double disc diffusion / synergy test.

Design: The bacterial strains were isolated from pus, sputum, blood, urine, pleural fluid, peritoneal fluid and cerebro-spinal fluid samples, obtained from both Nosocomial and outpatients.

Setting: The samples were obtained from patients admitted in oncology, post-operative surgical, kidney transplant center / urology wards and intensive care unit of Pakistan Institute of Medical Sciences as well as outpatients of the hospital.

Subject: Bacterial isolates, of family Enterobacteriaceae, were obtained from 200 Nosocomial and 200 outpatients (ambulatory). The isolates were sub-cultured, identified, and the double disc diffusion / synergy test was performed for detection of ESBLs.

Main outcome measures: Double disc diffusion / synergy test, for the detection of ESBLs production in Enterobacteriaceae.

Results: Prevalence of ESBLs in the Enterobacteriaceae was found to be 37.50% in Nosocomial and 06% in outpatient isolates. Highest prevalence was seen in *Klebsiella pneumoniae* (70%), followed by *Enterobacter cloacae* (33.33%) and *Escherichia coli* (28.57%) in case of Nosocomial isolates while in case of out-patient (ambulatory) isolates, the *Enterobacter cloacae* are the most prevalent ESBLs producers (8.33%).

Conclusions: Prevalence of ESBLs among the bacteria of family Enterobacteriaceae was higher in isolates obtained from Nosocomial patients as compared to out-patient (ambulatory) isolates. Such type of antimicrobial resistance appears to be particularly influenced by irrational use of antibiotics. To overcome this problem, the combined competencies of clinicians, microbiologists and the infection control team are needed.

KEY WORDS: Extended-spectrum ß-lactamases, Enterobacteriaceae, Klebsiella pneumoniae, Enterobacter cloacae, Escherichia coli.

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INTRODUCTION

Antibiotic resistance in isolates of Enterobacteriaceae and other Gram-negative bacilli is emerging in many parts of the world as a major threat to successful therapy of infections in hospitals. ß-lactamases of Gram-negative bacteria are the most important mechanism of resistance against ß-lactam drugs. Other enzymes include aminoglycoside modifying enzymes, chloramphenicol acetyl-transferase, erythromycin esterases[1]. Plasmid–mediated ß-lactamases, are more important clinically as these can be transferred between various species of Gram-negative bacilli. These enzymes

are called extended-spectrum ß-lactamases (ESBLs). ESBLs can confer resistance against all ß-lactam drugs (penicillins, 1st, 2nd and 3rd generation cephalosporins, monobactams except carbapenems and cephamycins[2].

Many ESBL producing strains of Enterobacteriaceae do not show resistance to newer cephalosporins or aztreonam in routine susceptibility tests. Therefore, a clinical microbiology laboratory must not rely solely on routine susceptibility tests but should also use a more accurate method of detecting ESBLs[3].

The aim of the present study was to determine the prevalence of ESBLs among the members of family Enterobacteriaceae isolated from Nosocomial and out-door patients, with double disc diffusion test used routinely in clinical microbiology laboratory to detect ESBLs in these strains.

MATERIALS AND METHODS

Microorganisms

Bacterial isolates, of family Enterobacteriaceae, were obtained from 200 nosocomial and 200 outpatients (ambulatory). The isolates were sub-cultured, identified, and the double disc diffusion test/double disc synergy test was performed for detection of ESBLs. Samples from oncology, post-operative surgical, kidney transplant center/urology wards and intensive care unit were selected for study. The bacterial strains were isolated from pus, sputum, blood, urine, pleural fluid, peritoneal fluid and cerebro-spinal fluid.

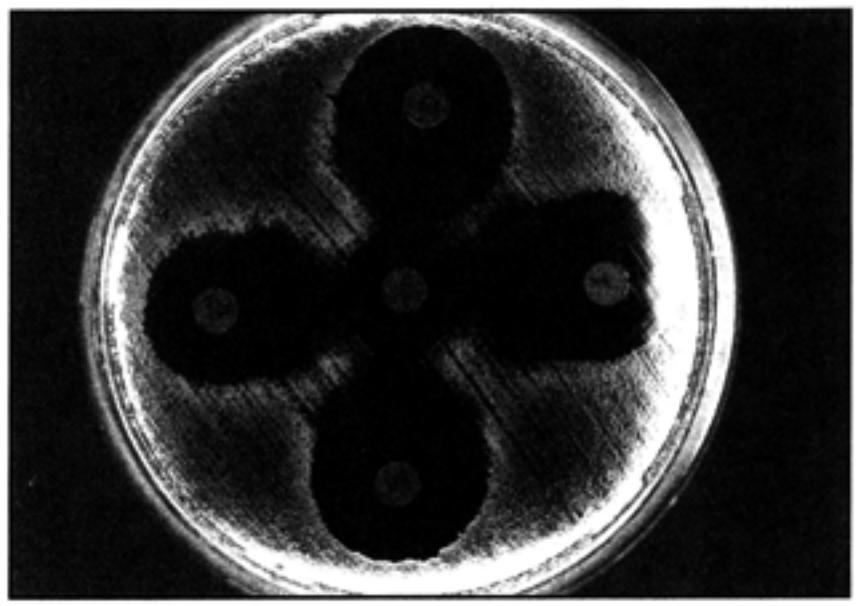
Screening for ESBL production

Strains were screened for ESBL by using disc diffusion technique. The following anti-microbial agents and break point diameters are indicators of ESBL; ceftazidime (<22mm), cefpodoxime (<22mm) and aztreonam (<27mm). Reduced zones around discs of cefotaxime (<22mm) or ceftriaxone (<25mm) may also indicate ESBLs but are less sensitive indicators (NCCLs, 1998).

Special test of ESBL detection

In the Double Disk Diffusion Technique by Jarlier[4] a single, separated colony of he test organism is picked with the help of wire loop and emulsified in 0.9% normal saline solution in a test tube. Matched the turbidity of test organism with 0.5% McFarland's Standard. The suspension of the test organism is spread on the Mueller-Hinton agar surface with the help of cotton swab soaked in suspension tube. Placed a disc of co-amoxiclav (20µg amoxicillin/10µg cavulanic acid) in the center of lawn of test organism on agar surface. Then placed the discs of cefotaxime, ceftriaxone, ceftazidime and aztreonam (30µg each) around the disc of co-amoxiclav. These discs were arranged in such a way that the distance between the central disc and surrounding discs should be approximately 30mm. Zones of inhibition around 3rd generation cephalosporin discs and aztreonam were observed after overnight incubation. If the inhibition zone around one or more cephalosporin discs and aztreonam was extended on the side nearest to the co-amoxiclav disc, the organism showing this synergism was ESBLs producer. If there was no extension of zones, then repeated the test by reducing the distance between the co-amoxiclav and cephalosporins and aztreonam discs. Then observed the zones of inhibition again on the next day. If there is no extension of zones of 3rd generation of cephalosporin and aztreonam towards co-amoxiclav disc, the organism will be considered as ESBLs non-producer[4].

Fig: Detection of ESBL
Double Disc Diffusion Technique



Key: AMC-Amoxycillinand clavulanic acid. CAZ-Ceftazidime. ATM-Aztreonam, CTX-Cefotaxime, CRO-Ceftriaxone,

RESULTS

Two hundred Nosocomial isolates of Enterobacteriaceae were collected from different wards and were tested for ESBLs production. Out of these 200 nosocomial isolates, 75 (37.5%) were ESBL producers. Out of these ESBL positive isolates, 45 (60%) were obtained from urine, 25 (33.33%) from pus and other body fluids, 05 (6.67%) from blood, while no ESBL positive isolate was obtained from CSF as shown in Table-I. Among individual species, prevalence of ESBL was highest in Klebsiella pneumoniae, 35 out of 50 (70%) isolates, followed by Enterobacter cloacae, 10 out of 30 (33.33%). ESBL was also isolated in 20 isolates of Escherichia coli, 06 of Proteus mirabilis and 04 isolates of Citrobacter freundii as shown in Table-II.

About 200 isolates of Enterobacteriaceae were collected from out-door specimen during the study period. Out of these 200 out-door isolates, 12 (06%) were ESBL producers. Among various species, prevalence of ESBL was highest in Enterobacter cloacae, i.e., 02 out of 24 isolates (8.33%) followed by Klebsiella pneumoniae and then Escherichia coli as shown in Table-III. The prevalence of ESBL producers was highest in pus/fluids (9.43%) and urine (7.69%) as shown in Table-IV.

Table-I: Distribution of ESBL positive nosocomial isolates in various specimens (n=75)

Samples	*Total*	*ESBL (+)*	*Percentage*
Urine	100	45	60
Pus/Fluid	80	25	33.33
Blood	15	05	6.67
CSF	05	00	00
TOTAL	200	75	37.5

Table-II: Distribution of ESBL positive nosocomial isolates in various species (n=200)

Isolate	*Total*	*ESBL (+)*	*Percentage*
E. coli	70	20	28.57
K pneumoniae	50	35	70
Ent. Cloacae	30	10	33.33
P. mirabilis	20	06	30
P. vulgaris	10	00	00
Cit. freundii	20	04	20
TOTAL	200	75	37.5

Key: E. coli, Escherichia coli; K. pneumoniae, Klebsiella pneumoniae; Ent. cloacae, Enterobacter cloacae; P. mirabilis, Proteus mirabilis; P. vulgaris, Proteus vulgaris; Cit. freundii, Citrobacter freundii.

Table-III: Distribution of ESBL positive outpatient (ambulatory) isolates in various species (n=200)

Isolate	*Total*	*ESBL (+)*	*Percentage*
E. coli	80	05	6.25
K pneumoniae	41	03	7.32
Ent. Cloacae	24	02	8.33
P. mirabilis	30	01	3.33
P. vulgaris	10	00	00
Cit. freundii	15	01	6.67
TOTAL	200	12	06

Key: E. coli, Escherichia coli; K. pneumoniae, Klebsiella pneumoniae; Ent. cloacae, Enterobacter cloacae; P. mirabilis, Proteus mirabilis; P. vulgaris, Proteus vulgaris; Cit. freundii, Citrobacter freundii.

Table-IV: Distribution of ESBL positive outpatient (ambulatory) isolates in various speciemen (n=200)

Specimen	*Total*	*ESBL (+)*	*Percentage*
Urine	91	07	7.69
Pus/fluid	53	05	9.43
Blood	05	00	00
Ear Swab	28	00	00
HVS	11	00	00
Sputum	12	00	00
TOTAL	200	12	06

DISCUSSION

Prevalence of ESBL producing strains among various species of Enterobacteriaceae varies world wide from <1% to 74%[3]. In our study, overall prevalence of ESBLs in Nosocomial isolates was found to be 37.5%. It is much higher than that observed in a teaching hospital in France, 2.7% in 1990 and 2.9% in 1994[5].

In the present study, out of 200 nosocomial isolates, most of the ESBL producers were isolated from urine (about 60%). Highest incidence of ESBL positive isolates in urine has also been reported by Jarlier et al. (1988). The results show that 6.67% ESBL producers were isolated from blood while Jarlier et al. 1988 has reported 18% of the ESBL positive organisms from blood. This is probably due to the reason that in our set-up, pus specimens are usually not accompanied by blood specimens. Our clinicians usually send samples from local site of infection.

Prevalence of ESBL producing strains in various species of Enterobacteriaceae varies in different countries and different hospitals. Usually one of the three species (Klabsiella pneumoniae, Escherichia coli, Enterobacter cloacae) is at the tope of the list. Philippon[6] et al. reported 9% of all Klebsiella pneumoniae isolates to be ESBL producers in 16 different French hospitals and 8.6% ESBL producing Klebsiella pneumoniae were reported in USA[2]. While in our study a very high percentage of Klebsiella pneumoniae (70%) were found to be ESBLs producers whereas Chanal[5] et al. reported the study which they carried out in 1990, Enterobacter was most prevalent ESBL producer followed by Klebsiella pneumoniae. In 1994 the same investigators found Klebsiella pneumoniae to be more prevalent. In one study carried out from July 1990 to July 1991, Klebsiella pneumoniae was found to be the most prevalent ESBL producer[7]. As compared to Klebsiella pneumoniae, Escherichia coli usually less commonly produces ESBLs. In our study, a significant amount of Escherichia coli, i.e., 28.57% was obtained from specimens from different wards. In one study in France <0.2% of Escherichia coli were ESBL producers[5]. In another study in USA, 1.3% of Nosocomial isolates of Escherichia coli were ESBL producers[2]. In the present study ESBLs were also detected in a significant proportion in other species, e.g. Proteus mirabilis (30%) and Citrobacter freundii (20%).

The results show that 06% isolates from outpatients were ESBL producers. This is a very high frequency as ESBLs are rarely seen in outpatients[8]. In fact studies for ESBL prevalence are usually done on Nosocomial isolates only, as all the risk factors for ESBL production are related to hospital stay. About 7.69% ESBL producers in our study were isolated from urine, while 9.43% were isolated from pus/fluids. Another explanation could be that in our society extended-spectrum cephalosporins are used in outpatients. Sometimes patients are having long term indwelling urinary catheters and they get these catheters changed from different hospitals. Isolation of ESBL producers from pus could be explained on similar grounds. Such patients may acquire these isolates from some hospital while undergoing some minor surgical procedures in outpatient e.g. (incision and drainage of an abscess, change of dressing etc.).

Admission to a nursing home, excessive antibiotic exposure (especially to ceftazidime) extended hospital stay, recent surgery, admission to an ICU and instrumentation have been identified as risk factors for the selection of ESBL producing strains[7,9]. In fact, the risk factors for infection by ESBL-containing organisms are similar to those for other Nosocomial pathogens such as methicillin-resistant staphylococci and vancomycin-resistant enterococci, and also include prior antibiotic administration, arterial, venous and urinary catheters, prolonged length of stay, stay in an ICU, and severity of illness[10]. Studies have shown that a risk factor for infection is also the prior use of antibiotics, especially cephalosporins[11,12]. These organisms are spread between patients in a manner similar to that of other Nosocomial organisms, namely through the contaminated hands and equipment of healthcare workers[13].

Apart from ICUs ESBL producing strains have also been found in patients in general wards and nursing homes. Use of 3rd generation cephalosporins is the most important factor for acquiring ESBLs. Some investigators have identified abdominal surgery as the major risk factor[9]. The length of stay in ICU is also important. In one study more than half of the patients were colonized after 30 days of stay in hospital[14].

There are certain other factors that participate in the development of resistance. These include: a) transfer of resistance genes among bacteria which transform susceptible strains to resistant ones, b) dosage and types of antibiotics which cause the selection pressure to certain species of bacteria, c) level of organization and strict adherence to hygienic and anti-epidemic regimen starting with the entry of patients into the hospital. Analyses are necessary to check whether the patient brings resistant bacteria with a transferable resistance (with ESBLs) into the hospital[15].

Preventive measures would be strictly applied to stop the clonal spread of resistant strains among the patients and/or hospital environment, which occurs if these strains have such opportunity. Last, but not least to be considered is the dosage and administration of anti-bacterials, especially in post-operative prophylaxis in intensive care units and application of anti-bacterials to patients when necessary[15]. Prevention and control measures are important because of the multi-resistant nature of these pathogens. Since this type of anti-microbial resistance appears to be particularly influenced by antibiotic use, antibiotic control measures may also be very important in controlling the spread of ESBLs[16]. In medical practice, the development of resistance poses various problems like that for the physician who must prescribe an active antibiotic that does not select resistant mutants, problem of detecting resistances such as the production of extended-spectrum ß-Lactamases and a problem for the control of infection to limit the dissemination of multi-resistant bacteria. To overcome these problems, the combined competences of clinicians, microbiologists and the infection control team are needed[17].

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