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Full Length Research Paper

Prevalence and comparison of Beta-lactamase producing *Escherichia coli* and *Klebsiella* spp from clinical and environmental sources in Lahore, Pakistan

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Beta-lactamase producing *Enterobacteriacea* is a significant problem all over the world. There is lot of research on worldwide distribution of this enzyme. We studied the prevalence of ESBL producing *Escherichia coli* and *Klebsiella* isolates from clinical and environmental sources. For selection of ESBL isolates criteria of standard clinical laboratory standard institute was followed. Result showed that *E. coli* is most common in ESBL producer following *Klebsiella, Enterobacter* and *Citrobacter*. *E. coli* is more prevalent in both hospitalized and community acquired infections. Females were more prone to infections caused by ESBL producers. Urinary tract infection was more common in females infected by ESBL producing isolates while pus was the commonest site of infection in males suffering from ESBL producing infections. Males of older age and females of reproductive age were more prone of infections caused by ESBL producers. In environmental source, sewage was most prevalent source of ESBL producers following soil and standing water. This gives the idea that the prevalence of this superbug in this part of the world is very high which needs special attention of the society.

Key words: Betalactamase, Pakistan, Clinical and environmetal isoltes.

INTRODUCTION

Beta-lactamase producing *Enterobacteriaceae* has become a global problem. The rate of incidence and type of beta-lactamase enzyme varies in different worldwide geographical locations. From more than 30 countries, research related to Extended Spectrum Beta-Lactamases (ESBLs) has been published which presents worldwide distribution of ESBLs (Paterson and Bonomo, 2005; Coque et al., 2008). Beta-lactamase producing bacteria have property of enzyme production and it is reported that if not properly diagnosed inappropriate antibiotics pattern results. This problem also leads to wastage of precious time of patients and a great drawback economically. ESBL producing antibiotic resistant environmental bacteria are also consistently increasing worldwide due to excessive discharge of antibiotics in environment. Consistent excessive use of antibiotics exerts pressure on bacterial evolution, hence producing modified antibiotic resistant bacteria. Antibiotic resistant gene can occur in nonpathogenic bacteria, which can be transferred via lateral gene transfer. World Health Organization (WHO) announced antibiotic resistance as one of the three major public health threats of this century (Lachmayr et al., 2009).

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MATERIALS AND METHODS

Clinical bacterial strains were isolated in Citilab and Research Center from June, 2007 to December, 2008. All clinical specimens were processed according to standard operating procedures (NCCLs, 2004). All samples were inoculated on blood agar and

Table 1. Source of beta-lactamase producing clinical isolates.

Clinical isolate	Specimens (n=300)				
	Urine	Blood	Pus	F. tips	Others
E. coli	145	15	55	25	20
Klebsiella species	14	7	7	6	6

Total *E. coli* n=260, *Klebsiella* n = 40.

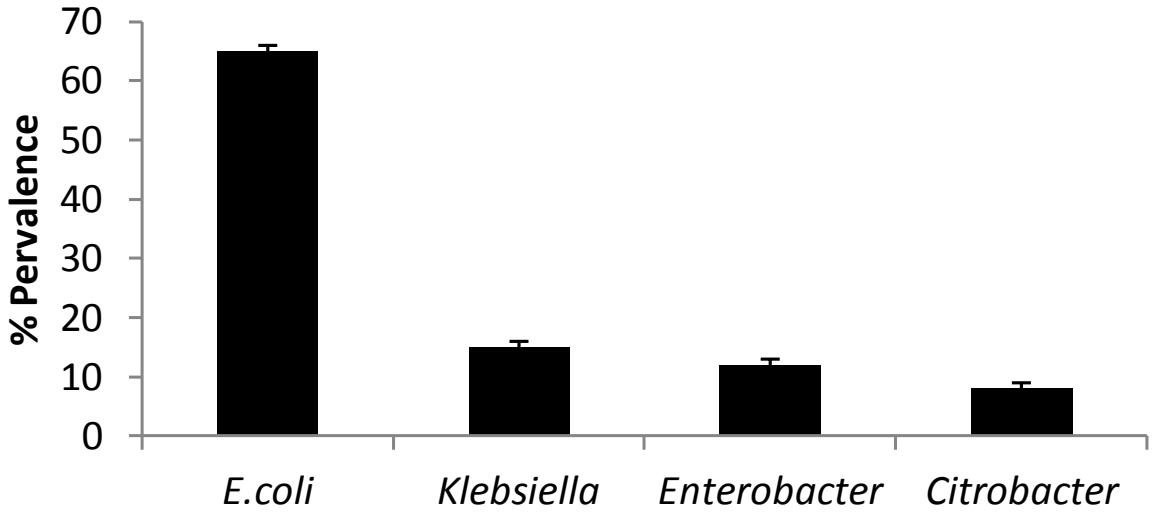


Figure 1. Prevalence of lactose fermenting *Enterobacteriaceae.*

McConkey agar. Only infection causing positive isolates were processed for Gram staining. Lactose fermenting gram-negative bacteria was characterized by colony morphology and biochemical tests. Confirmation of identification was performed by using 20E API (BioMerieux). Data were reported in line with the standard criteria of the Health Protection Agency (HPA, 2006). Environmental ESBL bacteria were isolated from different environments such as sewage, pond water, hospital soil. Gram-negative lactose fermented bacilli were isolated using biochemical parameters and 20E API as performed for clinical isolate. ESBL producers were confirmed by double disc synergism test using augmentin, ceftazidime, cefuroxime and azotranum.

RESULTS

Out of 1018 *Enterobacteriaceae* members, 662 *E. coli*, 153 *Klebsiella* spp, 122 *Enterobacter* and 81 *Citrobacter* were isolated. Clinical samples included urine, blood, wound swabs and others (Table 1). A total 300 ESBL bacterial strains were positive for beta-lactamases, where 260 were *E. coli* and 40 were *Klebsiella* spp (Figure 1). Approximately 39.27% *E. coli* and 26.10% *Klebsiella* species was found to express ESBLs. The most prevalent infection caused by beta-lactamase producing gram-negative lactose fermenting isolates was urinary tract infection. Urine sample infections were the most common followed by pus or wound swab and foley catheter tips, an artificial device used in hospitalized patients who are unable to urinate normally. Following these, blood was the common sample for which beta-lactamase producing bacteria were causative agents of septicemia. Other sample sites included high vaginal swabs, tracheal secretion, sputum, bronchial washing, oral tip etc. Beta-lactamase producing isolates were mainly in several population attributes, sample size and hygienic conditions of patients.

All data were analyzed according to *E. coli* and *Klebsiella* spp separately. Regarding gender classification in the case of beta-lactamase producing *E. coli*, females (60%) had a higher incidence as compared to males (40%). Similarly, in case of *Klebsiella,* females (55%) and males (45%) were effected (Figures 2a and b). Comparison of hospitalized and community acquired infections presents that in both cases >55% *E. coli* caused infection following *Klebsiella* >45% (Figures 3a and b). Here data of *E. coli* and *Klebsiella* was arranged according to source of specimens. However, in the case of pus swabs, males were more affected with 71 and 69% of *E. coli* and *Klebsiella*, respectively. In blood samples, infection with *E. coli* was 60 and 50% *Klebsiella* effects males (Figures 4a and b). Foley tip specimens

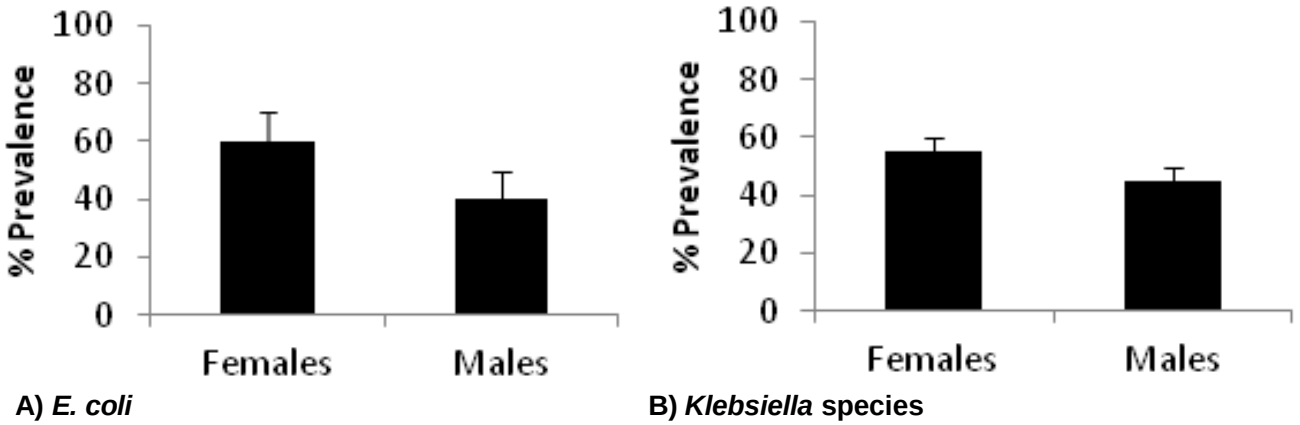


Figure 2. Prevalence of beta-lactamase producing *E. coli* and *Klebsiella* according to gender classification.

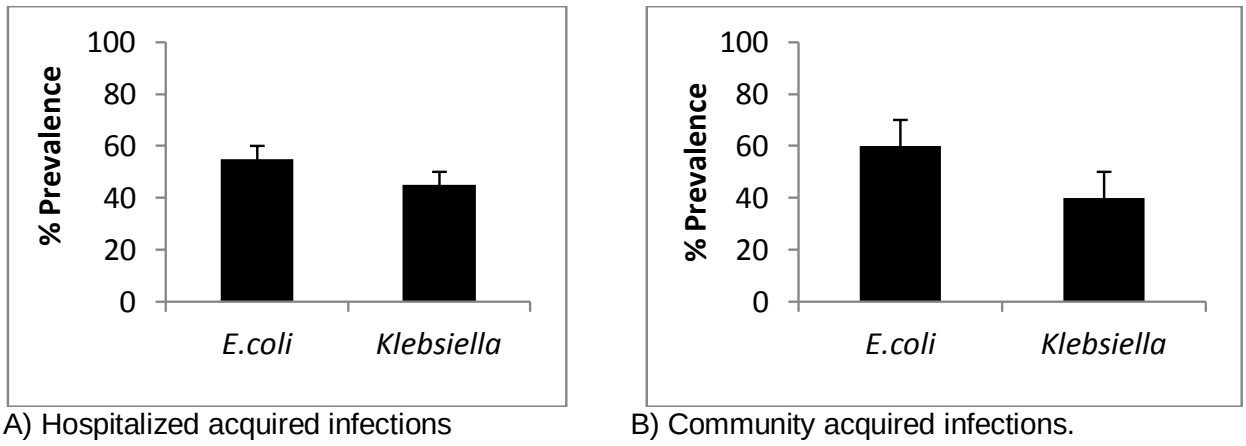


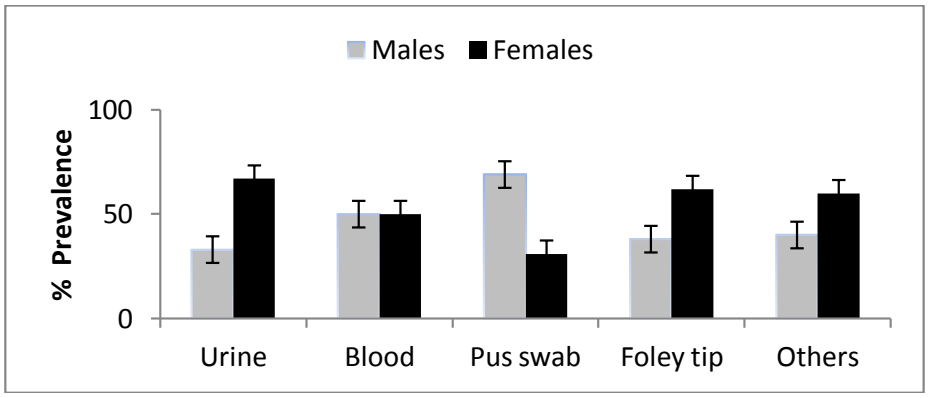
Figure 3. Prevalence of beta-lactamase producing *E. coli* and *Klebsiella* in (A) hospitalized and (B) community acquired infections.

were from hospitalized patients only whereby 29% males and 71% females were affected with *E. coli* (Figures 4a and b). However, in *Klebsiella* foley tip infections, 62% were from females and 38% from males. Other specimens had high infection rate in females 60% whereas in *Klebseilla* it was 50%.

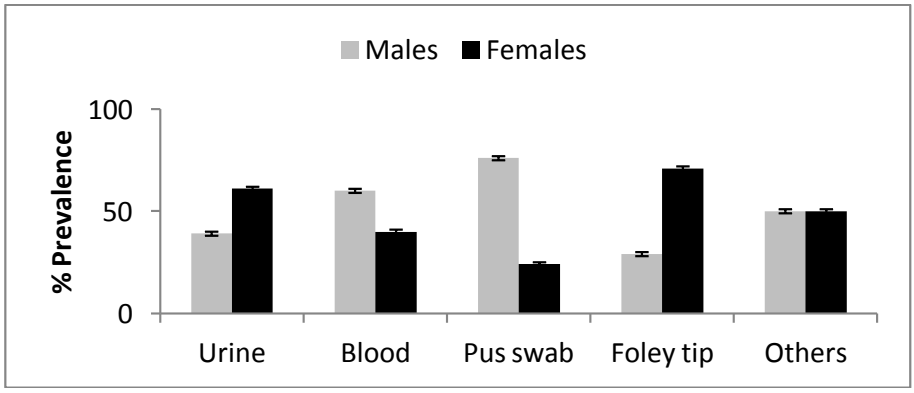
Prevalence of beta-lactamase producing *E. coli* was high in age groups 50+ followed by 60+ and 70 + in males. While in females, it was more prevalent in 30+, 50+ and 40+. Beta-lactamase producing *Klebsiella* had high frequency in age groups between 50+ and 70+ in males. On the other hand, high frequency in females was in age groups of 70+ and 20-30+ (Tables 2a and b). Prevalence of environmental *E. coli* and *Klebsiella* were evaluated. There were three major sources of ESBL isolates which includes sewage, pond water and hospital soil. Sewage was collected all over Lahore for isolation of bacteria. Similarly, pond water/standing water and hospital soil were collected for isolation of ESBL bacteria. Of 100 ESBL isolate (E1-E100) 87 were *E. coli* and 13 were *Klebsiella* spp. Result presents that ESBL *E. coli* and *Klebsiella* spp were commonly present in domestic sewage (87.3 and 76.9%) following hospital soil (15.3 and 8%) and pond water (7.6 and 4.5%) respectively (Table 3). Hospital soil had ESBL producing bacteria significantly as compared to other soil areas. That's why only hospital soil had ESBL producing bacteria.

DISCUSSION

The prevalence of ESBLs is increasing rapidly. This creates an alarming situation because the prevalence of ESBL, type of enzyme, gender and age groups in which ESBLs present varies in different geographical areas. Members of *Enterobacteriaceae* are the most common causative agent of nosocomial and community bacquired infections (Coque et al., 2008). Only most prevalent



A) *E. coli*



B) *Klebsiella* species

Figure 4. Frequency of specimen sites in different genders caused by beta-lactamase producing (A) *E. coli.* and (B) *Klebsiella* spp.

Table 2a. Prevalence of beta-lactamase producing *E. coli* in males and females of different age groups.

Age group	Males no.	Percentage	Females no.	Percentage
0+	6	5.0	7	4.9
10+	11	9.3	10	7.0
20+	6	5.0	14	9.8
30+	20	16.9	30	21.1
40+	9	7.6	20	14
50+	23	19.49	27	19
60+	20	16.9	14	5.8
70+	20	16.9	17	11.9
80+	3	2.5	2	1.4
90+	-	-	1	0.7
Total	118	100	142	100

Enterobacteriaceae members were considered here such as *E. coli* and *Klebsiella.* The major reason of this spread of infection is likely due to inappropriate use of antibiotics and self-medication. Occasionally, other source like hospital cross infections and animals may be involved in the spread of infection (Jitsurong et al., 2006). There are many reports, which showed ESBLs prevalence from Latin America, Asia and also North America(Babypadmini et al., 2004). Here 55% *E. coli* and 45% in *K. pneumonia* were ESBL producer in the case of hospitalized

Table 2b. Prevalence of beta-lactamase producing *Klebsiella* in males and females of different age groups.

Age group	Males no.	Percentage	Females no.	Percentage
0+	-	-	-	-
10+	1	5.5	1	4.5
20+	2	11.11	5	22.7
30+	2	11.11	4	18.18
40+	1	5.5	3	13.6
50+	6	33.33	2	9.0
60+	1	5.5	1	4.5
70+	5	27.7	6	27.2
80+	-	-	-	-
90+	-	-	-	-
Total	18	100	22	100

Table 3. Environmental sources of bacterial isolate.

Environmental isolate	Sewage no.	Pond water/standing water no.	Soil no.
E. coli	76	14	7
Klebsiella species	10	1	2

Total n =100, *E. coli* n=87, *Klebsiella* n =13.

infections and 60% *E. coli* and 40% *Klebsiella* in the case of community acquired infections. Elsewhere, 30% ESBL-producing *E. coli* was detected in study at Aga Khan University in 2003 (Jabeen et al., 2003). Beta-lactamase producing isolates were classified by specimen site. It was observed that *E. coli* ESBL is more common in all sites of specimens. *E. coli* is a very common species that can live harmlessly in gut as a commensal, though may be more dangerous when it produces beta-lacatamases (Jabeen et al., 2003). Categorization of clinical ESBL infection sources showed that urine is a most common source of this infection. The majority of UTI infections (Figure 3) were community-acquired infections. Although there were reports, which gives an idea that *E. coli* is the leading cause of UTI in hospital-acquired infections the actual percentage was less than community-acquired infections (Lachmayr et al., 2009). In Iran, UTI causing ESBL producers were in high frequency. A study was conducted in a 1000-bed tertiary care hospital Iran showing that 21% *E. coli* and 12% *Klebsiella* were ESBL producer as causative agent of UTI (Behroozi et al., 2010). Even in Japan the increased frequency of UTI caused by ESBL producers were observed in 2006 (Muratani et al., 2006). Here beta-lactamase producing isolates were also classified according to gender. This data showed that infections in females might be more complex. Unfortunately, in our country there are limited healthcare facilities especially in rural areas, which may be the reason as to why the frequency of multidrug resistance is higher as compared in developed countries. In India, the situation is similar to Pakistan because of socio-economic status. Iran and China exhibit similar prevalence, phenotypic and genotypic characteristics of ESBL producers and have much better health care facilities as compared to Pakistan. Antibiotic resistance is also related to age groups because in very young age or old age groups when a patient becomes immune-compromised there are greater chances that they suffer from infections Table 2a and b). Such infections are of many types but in patients with low immunity it is difficult to treat such infections hence, the risk in becoming more serious or non-treatable (Muratani et al., 2006). This is the leading cause of ESBL infection in old age as suggested by data. Data revealed that in Pakistan multidrug resistance was more common in the age group of 50+ in both males and females (Bashir et al., 2008).

Another study confirms the finding whereby old age and antibiotic resistance appear to be correlated (Lindback et al., 2010). Especially UTI and vaginosis are extremely common in pregnancy (Yudin et al., 2008). In males, urethritis and prostatitis are very common infections. Prostatitis often occurs in age group of 40+ and may be associated with the diagnosis of prostate cancer (Reyes et al., 2009).

Environmental ESBL isolates were examined for better understanding of the risk of dissemination of ESBL bacteria to humans from different environmental sources. Environmental antibiotic resistant gene can be transferring their resistance both by lateral and horizontal gene mechanism. The significance of clinical ESBL is

well known and there is lot of literature on this subject. Although, significance of antibiotic resistance bacteria is known but there are few studies on environmental antibiotic resistant bacteria especially ESBL. ESBL producing gene can be transferred from animals to humans and even from other environment to humans. Hospital environmental bacteria have many ESBL isolates, which in turn become epidemics for hospital and society. Especially in ICUs where there were many reports about environmental contamination with ESBL *Enterobacteriaceae* isolate ranging from 5 to high to 26% (D'Agata et al., 1999; Kac et al., 2004). Isolation of ESBL producing environmental isolates is a difficult task. Many screening procedures are required before picking the particular isolate from mixture of bacteria. Two types of drugs such as ampicillin, cetazidime or cefotoxime was used as supplement for initial screening of ESBL isolates. In gram-negative lactose fermenter selected from three major sources, it was found that 29% *E. coli* and 17% *Klebsiella* spp produces ESBL. Pond water is also becoming a major source of ESBLs bacteria. Similarly, river water also had many diverse bacteria in reasonable amount that can produce ESBL. This is a potential problem and alarming situation in the spread of ESBLs throughout the community as spread is easy via water sources (Lu et al., 2010). Sewage is the major source of contamination for spread of antibiotic resistant bacteria. Maximum number of ESBL bacteria was isolated from sewage following hospital soil and pond water. This data suggest that ESBL environmental bacteria are frequently present in our environment that needs special attention of the society.

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