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ORIGINAL ARTICLE

Genomic Analysis of Highly Virulent *bla*CTX-M, *bla*SHV and *bla*TEM Genes in Resistant Strains of *E.coli* and *Klebsiella*: an emerging threat

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ABSTRACT

Background: The term antimicrobial resistance refers to the ability to resists the effects of drugs formally used to treat them and this term relates only to bacteria becoming resistant. Microorganisms that are resistant to multiple drugs are known as multidrug-resistant bacteria.
Aim: To investigate the prevalence of SHV, TEM and CTXm genes from *E.coli* and *Klebsiella* isolated from patients of Lahore, Pakistan.
Methods: Patients with prolonged hospital stay were enrolled in this cross-sectional study with 60 samples comprising *Klebsiella pneumoniae* and *Escherichia coli* (Ctx, Cro, Caz resistant) were identified in clinical specimens. To assess susceptibility, the disc diffusion method was applied with eight antibiotic panel of cephalosporin 3rd generation. A Double disc, combined disc test was used to identify the ESBL-producing bacteria. By real - time PCR, The presence of the genes encoding blaTEM, blaSHV, and blaCTXm was tested in ESBL positive isolates as well as additional isolates with MICs of less than 4g/mL for ceftazidime, cefotaxime, ceftriaxone, and aztreonam (PCR).
Results: The frequency of *E. coli* and *Klebsiella* bacteria was found in 59% and 41% of the 60 samples, respectively. According to the data, 23 isolates (16.37%) were multidrug resistant, and 7(6.89%) were ESBL-positive. At least one of the antibiotics ceftazidime, ceftriaxone, or cefotaxime was resistant to 30(25.86%) of the isolates. The ESBL genes were sequenced to corroborate the PCR result.
Conclusions: Among E. coli and *Klebsiella* bacteria obtained from patients, blaTEM-116 was the most frequently isolated ESBL gene, followed by Shv and Ctxm.
Keywords: Antibiotic Resistance, Beta- Lactamases, *E. coli*, *Klebsiella pneumonia*

INTRODUCTION

E.coli and *Klebsiella* are Gram-negative bacteria found in water and soil[1] as well as on human skin and gastrointestinal tract flora[2] that cause nosocomial infections in immunocompromised cancer patients[3], cystic fibrosis, burns, and other disorders. The majority of standard antibiotics are ineffective against these bacteria[4]. The most common ESBLs are classified into three groups: TEM, SHV, and CTX-M[5]. *E.coli* and *Klebsiella* are well-known for their resistance to third-generation cephalosporins[6]. They have developed a number of extended spectrum -lactamases (ESBLs), which allow bacteria to resist extended-spectrum cephalosporins including cefotaxime, ceftriaxone, and ceftazidime, recorded more frequently in recent years[7]. Traditionally, ESBLs were generated from the main enzymes TEM and SHV[8]. ESBLs of the CTX-M type, in particular, became highly widespread last year[9]. ESBLs have been categorized into nine distinct structural and evolutionary groupings that are rapidly expanding in healthy persons. TEM and SHV were the most prevalent types. Nonetheless, the CTX-M type is more widespread in other countries[10].

The goal of this research was to isolate and identify and then Genomic analysis of highly virulent SHV, TEM and CTX-M Genes in Resistant Strains of E.coli and *Klebsiella*

METHODS

Sample Collection and Identification: Clinical specimens such as blood, urine, and pus were used to isolate samples. On a printed card, all patients were provided instructions for proper specimen collection[11]. Traditional biochemical tests were used to identify all of the bacteria isolated from urine in this investigation[12]. The Clinical and Laboratory Standards Institute (CLSI)[13] guidelines was used to carry out this procedure.
Antimicrobial susceptibility tests: The Kirby-Bauer method, based on Clinical and Laboratory Standards Institute (CLSI) guidelines from 2018, was used to test all isolates for antibiotic susceptibility[14]. Cefotaxime (30g), ciprofloxacin (30g), and ceftazidime (30g) were the antibacterial discs utilized (MAST DISKSTM, UK). If the inhibitory zone diameter is 5mm bigger with clavulanic acid than without[14], the test is positive.
DNA Extraction and Polymerase Chain Reaction (PCR): The DNA Mini Kit had been used to obtain genomic DNAs (QIAGEN, Germany). We injected lysostaphin to the lysis buffer at a final concentration of 30 g/mL per the manufacturer's procedure for microbial species[13].
Polymerase Chain Reaction Product Sequencing: The entire set of 30 isolates' DNA was sequenced using a DNA sequencing kit and evaluated in an automatic DNA analyzer (Bioneer Company, Korea).
Statistical analysis: SPSS (PASW) version 18 software was used for descriptive data analysis.
Permission was granted by the institutional Ethical Review Board.

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RESULTS

In all of the above-mentioned samples frequency of occurrence of *E. coli*, *Enterobacter* and *Klebsiella* strains were 59%, 23%, 18% respectively. Total (n=60) samples were collected from Sir Ganga Ram Hospital Lahore. Most of them (n=36) were ESBLs positive and about (n=24) were ESBLs negative and their percentage was 60% and 40% respectively. In total 60 collected samples (n=25) were of urine, of which 64% were ESBLs producers and 36% were non-producers. Total (n=14) samples of blood were collected of which 57% are ESBLs producers and about 43% were non-producers. Total (n=14) samples of pus were collected of which about 64% were ESBLs positive while 36% were negative. There were (n=7) out of (n=60) samples were of sputum and their percentage is about 43% and 57% are ESBLs producers and non-producers respectively.

It was found that *E.coli* was 64%, 57%, 57% and 42% in samples of urine, blood, pus and sputum respectively. The results were found that 20%, 43%, 7% and 29% *Enterobacter spp.* were in samples of urine, blood, pus and sputum respectively. It was

estimated that about 16%, 0%, 36% and 29% of *Klebsiella spp.* were found in *samples* of urine, blood, pus and sputum respectively. A total of 60 different clinical samples was collected of which 36 were ESBLs positive and 24 were ESBLs negative (Table 1). The accession number and complete data of highly resistant genes is shown in table 1. Prevalence of SHV TEM and Ctxm genes however genomic analysis of antibiotic resistant genes in *E.coli* and *Klebsiella*.

DISCUSSION

Bacteria continue to be a significant threat to human health as a source of medical-related disorders[13]. Antibiotic resistance in *E.coli* q in microorganisms has emerged as a major therapeutic concern worldwide[14]. Resistance to extended-spectrum cephalosporins has recently emerged as a result of the usage of second and third generation cephalosporins[15]. The blaCTX-M gene has now become significantly more widespread over the globe within last year, far outscoring the ESBL genes blaSHV and blaTEM. Other genes linked to the blaCTX-M15 gene include blaSHV and blaTEM, genes which provides resistance to aminoglycosides and fluoroquinolones[14].

The blaCTX-M1 gene was revealed to be the major ESBL genes in clinical specimens of *Klebsiella pneumoniae*[15], *Salmonella enterica*[16], and Shigella spp.[17] in previous studies done in our country. Similarly, statistics on the growth of CTX-M15-producing Enterobacteriaceae in neighboring countries including Kuwait[18], Saudi Arabia[19] and Turkey[20] back up with findings. However, there is a paucity of information on the prevalence of blaCTX-M15 in *E. coli.* The much more common blaCTX-M variant in enteroaggregative *E. coli* (EAEC) and EPEC in Kuwait, as according Albert et al[21], was blaCTX-M28. The blaCTX-M1 family includes the genes blaCTX-M28 and blaCTX-M15. In the United Arab Emirates, the blaCTX-M15 has been the only model available[22].

In South America, *E.coli* strains harboring the blaPER-2 and blaTEM-116 Extended - spectrum beta genes have indeed been obtained from diarrheal kids[23]. *Klebsiella* was substantially more resistant to multiple antibiotics than Enterobacter cloacae in Nicaragua, according to Amaya et al[24]. CTX-M15 enzyme also was seen to be the most frequent kind of ESBL in *E. coli* isolates from children either with or without gastroenteritis[25]. Some Beta - lactamases (including such blaSHV-1 and blaTEM-1) derived from previous, broad-spectrum -lactamases [26]. Nearly half of something like the samples in this investigation have the blaSHV gene. Interestingly, in five isolates, the gene coexisted alongside blaCTX-M15. Non-ESBL producers make up the vast majority of SHV enzyme-expressing isolates. The blaCTX-M15 and blaTEM genes have been identified in just two isolates.

Additionally, since clavulanic acid induces large levels of AmpC -lactamases production, the combination of both AmpC - lactamases and ESBLs in the very same strain may result in a completely bogus phenotypic diagnostic method[27]. Molecular class A (e.g., KPC) was missing in the case of imipenem[28]. On the other hand, Blanco et al[29] discovered that CTX-M were more common[30]. Serogroups can shift over time, by location, and even within a single country[29-31,32]. MLVA has the potential to be a genotyping tool. In several European countries, it has also been used to genotype *E. coli* strains harboring unique CTX-M family groups[33], comparable to 33.3% gram-positive organisms[36], resulting in an increase in the incidence of gram-positive infections in the 1980s[37,38,39]. But nevertheless, between January 2007 and August 2013[42], conducted a comprehensive review on gram-negative pathogens that were frequently isolated[43], with one survey in Italy reporting an infection rate of 13.7% MDR gram-negative affiliated bacteremia and also another research in Pakistan reporting an incidence of 13.7% MDR gram-negative associated bacteremia.

Cephalosporins of the 3rd and 4th generations are indeed the first-line treatment options[44]. A appropriate first-line empiric antimicrobial should be utilised in this patient group[45]. Empiric antimicrobial treatment has reduced fatality rates for febrile immunosuppressed patients from around 21%[46] to 2–10%, dependent just on underlying medical condition[47,48]. Multi - drug resistant bacteria caused two - thirds of 91 cases of bacteremia in a prospective cohort study of 13 Brazilian HSCT sites[49]. A retrospective chart analysis of HSCT recipients was performed[50]. This findings are in line with a study published[51], which discovered that exposure to just about any broad-spectrum antibacterial agent may be enough to increase the likelihood of carbapenem-resistant Enterobacteriaceae, which is linked to increased comorbidities, death rates, and cost, especially in patients with hematological and biochemical diseases[52]. Furthermore, morbidity has been linked to limited early antimicrobial therapy in the case of antibacterial drugs bacteremia[53]. As a result, it's a good idea to keep an eye on the frequency of Multi - drug resistant strains in your area.

CONCLUSIONS

Finally, the importance of beta-lactamases, particularly ESBLs, in resistance to cephalosporin third generations' antibiotics in *E. coli* strains was highlighted in this study. It provided data regarding the current frequency of these variants as well as their genomic histories.

Conflict of interest: Nil

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