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Phenotypic Characterization of Extended Spectrum Beta-lactamase (ESBL) Producing Bacterial Isolates Recovered from Pharmaceutical Waste Sites

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ABSTRACT: Extended Spectrum Beta-Lactamases (ESBL) is one of the resistance mechanisms responsible for emergence of antimicrobial resistance (AMR). The occurrence of ESBL producing bacteria in the environment might serve as a resistance reservoir and pose a serious impact on human health. This study investigated the occurrence of ESBL producing bacterial isolates from pharmaceutical waste dumping sites. Soil samples were collected for the purpose and total 42 bacterial isolates were recovered and 17 different genera were identified. Preliminary screening was done using antimicrobial resistance to third generation cephalosporins and further confirmation by double disc diffusion synergy test (DDST). The isolates were most susceptible to cefotaxime (43%) followed by ceftriaxone (38%). In contrast, maximum resistance was observed for aztreonam (83%) and ceftazidime (80.9%) during *in-vitro* antibiotic cultural sensitivity assay. 51.51% isolates were confirmed as ESBL producers by DDST. Statistical analysis revealed the significant correlation between ESBL production and Multiple antibiotic resistance (MAR) score with $p<0.05$ for chi-square value of 38.44 at degree of freedom 5. All ESBL producers were having MAR score ranging between 0.8-1.0. Molecular characterization of ESBL genes to understand the specific resistance mechanisms and potential transmission pathways may help in a stronger understanding of the study. The absence of data on the potential impact of these ESBL-producing bacteria on human health and the lack of a longitudinal study design may limit the broader implications of the findings. Incidence of highly resistant bacterial strains in the environmental settings should not be overlooked and strict acquiescence to waste management guidelines is needed.

Keywords: Antimicrobial resistance, ESBL, cephalosporins, pharmaceutical waste, DDST.

INTRODUCTION

The future of antibiotics is in danger with the emergence of antibiotic resistance. In the past decade, a significant increase in the emergence of multiple drug-resistant (MDR) pathogens has been observed which has increased the demand for new antibiotics. Antibiotic resistance in bacteria is a worldwide problem and is linked to high morbidity and mortality rates (Akova, 2016). There are many ways by which bacteria can evade the effects of antibiotics like enzymatic degradation of antibiotics, effluence of antibiotics mediated by efflux pumps, modification of drug targets, reduced uptake of drugs etc. One such mechanism is the production of extended spectrum beta-lactamase (ESBL) enzymes. Extended spectrum beta-lactamases (ESBLs) are the enzymes produced by bacteriathat hydrolyze oxyimino beta-lactam antibiotics like cefotaxime, ceftriaxone, ceftazidime, and aztreonam but are still inhibited by beta-lactamase inhibitors like clavulanic acid (Kandasamy *et al*., 2016). They are serine β-lactamases belonging to the Ambler molecular and structural classification as class A. These resistance mechanisms can be inherent or acquired through genetic mutations or the transfer of resistant genes from other bacteria through horizontal gene transfer. Indiscriminate use of antibiotics is responsible for the evolution of antibiotic resistance (Banik *et al*., 2018). Infections associated with ESBL producers may range from minor infections such as urinary tract infections (UTIs) to more severe health conditions. The prevalence of ESBL-producing bacteria can vary depending on the specific environmental conditions. ESBL-producing bacteria are commonly detected in healthcare facilities such as hospitals and long-term care facilities (Martischang *et al*., 2021). These settings often provide a conducive environment for developing and spreading antibiotic resistance. However, ESBL-producing bacteria are not limited to healthcare settings and can also be found in the community. Studies have reported the presence of ESBLs in community-acquired infections, such as urinary tract infections and bloodstream infections (Abayneh *et al*., 2018). ESBL-producing bacteria have also been detected in healthy people, wild animals, and food-producing animals including poultry, swine, and cattle. They are also found in various environmental reservoirs, including water sources, sewage systems, and wastewater treatment plants (Cho *et al*., 2023). The emergence of

new and existing ESBL strains in our surroundings constitutes a serious threat in a clinical context. The occurrence or circulation of ESBL producers in our environment is hazardous as they give rise to multiple drug-resistance isolates (Salinas *et al.*, 2021). The emergence and spread of antibiotic-resistant bacteria highlight the need for prudent antibiotic use, infection control measures, and the development of new antimicrobial strategies to combat the growing threat of antibiotic resistance.

Resistance to broad spectrum beta lactams mediated by extended spectrum beta-lactamases, AmpC beta lactamases, and metallo beta-lactamase enzymes is an increasing problem worldwide. A high frequency of ESBL producers amongst *E. coli* was reported from a medical college of Himachal Pradesh in 2012 (Sood, 2012). Similarly, the occurrence of ESBLs, metallo-β-lactamases (MBLs), and AmpC-β-lactamases in clinical isolates of *Pseudomonas aeruginosa* was previously reported from Himachal Pradesh (Bharti *et al*., 2016; Bharti & Sharma 2014; Minhas & Sharma 2015). The emergence of such strains is of public health concern as such organisms may pose therapeutic challenges.

The prevalence of ESBLs producing bacteria in environmental settings is unknown in this region. Therefore, this current study aimed at phenotypically evaluating the prevalence of ESBL producing bacteria in pharmaceutical waste soils. This study will offer evidence on the reality of ESBL prevalence in the environment. The prevalence of such resistant strains in environmental settings is an alarming situation and proper attention has to be given to the management of such resistant strains to prevent their further transfer into the community. Appropriate antibiotic use and discard policies should be implemented in clinical as well as environmental settings so that the emergence of antibiotic resistance may be restricted.

MATERIAL AND METHODS

A. Study sites and sampling

Soil samples from waste dumping sites including effluent waste sites, solid waste sites, and waste water treatment sites of 14 pharmaceutical companies located in Parwanoo, Kalka, and Baddi areas of Himachal Pradesh were collected aseptically from at least 6 inches deep of soil with the help of a sterile spatula. All the collected samples were kept in a sterile zip-lock plastic bag and transported to the Microbiology laboratory, at Himachal Pradesh University and stored at 4°C. Bacterial isolation was carried out within 24-48 hours of collection.

B. Bacterial isolation and identification

Soil samples were serially diluted in 10-fold physiological saline and 1mL aliquots of appropriate dilutions (10^{-2}–10^{-6}) were inoculated and plated on nutrient agar (NA) medium. The plates were incubated under aerobic conditions at 37°C for up to 48 hrs. Morphologically distinct colonies were further re-streaked on nutrient agar plates (Himedia, Mumbai) to obtain pure cultures. Morphological characteristics were evaluated by microscopic analysis of Gram's-stained preparations and isolated bacteria were further identified biochemically in a systematic way following standard procedures (Varghese & Joy 2014). The findings were interpreted as per Bergey's Manual of Determinative Bacteriology, Volume 3 (Holt, 1994). The bacterial isolates were maintained on nutrient agar slants and sub-culturing was done regularly to maintain fresh cultures for the experiment. The purified colonies were stored in 40% glycerol stocks and kept at -20°C for further use.

C. Primary screening of isolates for ESBL production

Initial screening of all the bacterial isolates for their ability to produce ESBLs was performed by an *in vitro* antibiotic culture sensitivity assay using Mueller Hinton agar (MHA) following the Kirby–Bauer disc diffusion method. Antibiotic discs of cephalosporins class impregnated with different concentrations (Hi-Media, Mumbai, India) were employed: Aztreonam (30µg), Ceftazidime (30µg), Cefpodoxime (10µg), Cefotaxime (30µg), Ceftriaxone (30µg), and Cefuroxime (30µg). The lawn cultures of each bacterial isolate were prepared on MHA plates and the sensitivity discs were carefully placed on each plate. The plates were incubated at 37°C for 24 hrs (Bauer *et al*., 1966). Phenotypic antibiogram was prepared by measuring the zones of growth inhibition around each disc and the results were interpreted as Resistant (R), Intermediate (I) and Sensitive (S) as per the zone breakpoints mentioned in the Himedia catalogue 2023-24 based on latest standards of the Clinical and Laboratory Standards Institute (M100-S32). Only those isolates that were resistant to at least three or more antibiotics tested were confirmed further using DDST.

D. Confirmation by Double-disc diffusion synergy test (DDST)

ESBL production was further confirmed by a phenotypic detection method, i.e. a double disc diffusion synergy test. Synergism was determined between the two antibiotic discs: Cefotaxime (30µg) and Amoxyclav (20µg amoxycillin plus 10µg clavulanic acid). Plates were incubated at 37°C for 24 hrs and the diameters of growth inhibition zones were measured. The isolates showing well defined enhancement of the inhibition zone of cefotaxime in the presence of clavulanic acid with potentiation towards amoxyclav disc were considered probable ESBL producers (Clinical and Laboratory standard institute (CLSI), 2022).

E. Multiple Antibiotic Resistance (MAR) Index

MAR index was computed for all the isolates using the formula MAR = A/B, where "A" denotes the number of antibiotics to which the test isolate has shown resistance and "B" represents the total number of antibiotics tested (Roopa *et al*., 2023). Isolates with intermediate resistance (I) were taken as resistant (R) as a whole for calculating the MAR index.

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which isolate shown resistance (A)}}{\text{Total Number of antibiotics tested (B)}}$$

F. Statistical analysis

Chi-square (χ^2) test was applied to find the significant correlation between the production of ESBL phenotype and resistance to multiple antibiotics (MAR score) using test hypothesis as follows:

Null Hypothesis: There is no significant relation between ESBL production and Multiple antibiotic resistance (MAR) score.

Alternate Hypothesis: There is a significant relation between ESBL production and Multiple antibiotic resistance (MAR) score.

RESULTS AND DISCUSSION

A. Confirmation of bacterial isolates

Bacterial isolation and identification were performed in the research laboratory of the Department of Microbiology, Himachal Pradesh University, Shimla. From collected soil samples, forty-two (n=42) bacterial isolates belonging to 17 different genera were identified based on Gram staining and biochemical characteristics. The number distribution of bacterial isolates among genera is as follows: *Staphylococcus* spp. (n=5), *Klebsiella* spp. (n=5), *Pseudomonas* spp. (n=4), *Escherichia* spp. (n=3), *Bacillus* spp. (n=3), *Acinetobacter* spp. (n=3), *Corynebacterium* spp. (n=3), *Salmonella* spp. (n=3), *Citrobacter* spp. (n=2), *Proteus* spp. (n=2), *Shigella* spp. (n=2) and *Hafnia* spp. (n=2), *Enterococcus* spp. (n=1), *Enterobacter* spp. (n=1), *Micrococcus* spp. (n=1), *Streptococcus* spp. (n=1), and *Flavobacterium* spp. (n=1).

B. ESBL production (Primary screening)

Following in-vitro antibiotic susceptibility test, nearly all (83%) isolates were resistant to aztreonam followed by ceftazidime (79%), cefpodoxime (74%) and cefuroxime (67%). The bacterial isolates were highly susceptible to cefotaxime (43%) followed by ceftriaxone (38%) and cefuroxime (33%) (Fig. 1). 78.57% (33/42) isolates were preliminary screened as ESBL producers and remaining (9/42) were considered non-ESBL producers. One each isolate of *Citrobacter* spp. and *Klebsiella* spp. were found susceptible to all the antibiotics tested. On the other hand, the numbers of isolates among identified genera found resistant to all the antibiotics tested were as follows: *Escherichia* spp. (02), *Klebsiella* spp. (02), *Micrococcus* spp. (01), *Proteus* spp. (01), *Pseudomonas* spp. (02), *Salmonella* spp. (02), *Shigella* spp. (01), *Flavobacterium* spp. (01). In-vitroculture sensitivity assay results to various antibiotics are presented in Table 2. The isolate showing resistance to all the tested antibiotics is shown in Fig. 2.

C. Double Disk Diffusion Synergy Test (DDST)

ESBL as a mechanism of resistance was confirmed in 51.51% (17/33) of the primarily screened ESBL producers, using DDST. Isolate showing enhancement in the inhibition zone of cefotaxime and potentiation towards amoxyclav disc is shown in Fig. 3.

MAR Index. Antimicrobial susceptibility profile revealed that almost 55% (23/42) of the bacterial isolates had MAR score between 0.8-1.0, followed by 0.2-0.4 (14%) (Fig. 4). Two isolates had MAR score 0 because they were found susceptible to all the antibiotics tested Phenotypic antibiogram of the isolates along with MAR index is presented in Table 3.

Statistical analysis (χ^2 test). Statistically, the probability value (p-value) for a χ^2 of 38.44 with 5 degrees of freedom corresponds to a probability of less than 0.05. Hence, the null hypothesis was rejected and the alternate hypothesis was accepted. The bacterial isolates expressing ESBL phenotype had a significant relation with MAR score, as $p<0.05$ for calculated χ^2 of 38.44 (Table 4).

DISCUSSION

Extended-spectrum β-lactamase (ESBL) production is one of the major resistance mechanisms developed by bacteria to evade the mode of action of various antibiotic classes. ESBL producing strains have become a worldwide problem due to their ability to hydrolyze the β-lactam ring structure of 3rd generation cephalosporins and render the antibiotic ineffective. The treatment failures increased the out-of-pocket expenditure (OOPE) of patients and imposed catastrophic costs. Therefore, to safeguard the future of antibiotics, there is an urgent need for continued research, appropriate antibiotic use, and the development of innovative strategies to counteract and prevent antimicrobial resistance. In the present study, moderate occurrence (51.51%) of ESBL producers were reported among bacteria isolated from pharmaceutical waste soil. Similarly, a study from Poland reported ESBL positive *Enterobacteriaceae* among 19.8% of isolates recovered from municipal sewage water, their emission to the ambient air and the river receiving effluent from wastewater treatment plant (Korzeniewska & Harnisz 2013). Low incidence of ESBL may be due to multiple sampling sites with the same source i.e. municipal sewage waste, and a greater sample size as compared to our study. It may also come out to be higher in our study due to purposive source selection i.e. sampling from pharmaceutical waste dumping areas. Although, they have reported ESBL production among *Enterobacteriaceae* family only but our study revealed its occurrence in other bacterial families as well including *Pseudomonadaceae*, *Corynebacteriaceae*, *Moraxellaceae*, *Micrococcaceae*, *Flavobacteriaceae* and *Bacillaceae*. A comparatively low incidence (14.4%) of ESBL producers was reported among *E. coli* and *K. pneumonia* isolates from the river basin ecosystem in Tanzania (Kimera *et al*., 2021). A similar study from Himachal Pradesh, reported 95% resistance of *P. aeruginosa* isolates to at least one or more 3rd generation cephalosporins with 32.75% ESBL producers by the DDST method (Bharti & Sharma 2014). In our study, most bacterial isolates (83%) were resistant to aztreonam followed by ceftazidime (79%), cefpodoxime (74%), and cefuroxime (67%). In a similar study from Teaching hospital in Iran reported that 62.5% of bacterial isolates were resistant to third-generation cephalosporins (Tavajjohi *et al*., 2013). In a study conducted by Saleem *et al.* (2017) showed the frequency of ESBL-producing *E. coli* was 57.0% in healthy individuals, 53.0% in patients, 66.0% in cattle

faeces, 71.0% in sewage sludge,70.0% in raw meat, and 59.0% in chicken faeces. All of these isolates were resistant to cephalosporins and some of them were resistant to fluoroquinolones and meropenem. Another study by Sivaraman *et al.*, in 2021 showed simultaneous resistance to tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole by 28.1% of *E. coli* isolates and 86.7% of *K. pneumoniae* isolates from aquaculture farms and surrounding regions.

Cefotaxime was the only antibiotic for which least resistance was observed in our findings. In contrast, another study reported cefotaxime resistant *E. coli* between 1.8 and 4.8 (log10 CFU/mL) for cefotaxime antibiotic concentrations of 4 and 8 mg/L in the influent samples from wastewater treatment plant (Adegoke *et al.*, 2020). Although, the inhibitory and bactericidal effect of cefotaxime was higher among cephalosporins as evidenced by other recent studies (Gondane & Pawar 2023; Nath *et al.*, 1995).

In the present study, the correlation between ESBL production and MAR score was found statistically significant having p-value < 0.05 for χ^2 value of 38.44. The dataset used was for all the 42 isolates. However, it was also found significant if we include the dataset for isolates confirmed using DDST only i.e. 33/42. The p-value < 0.05 for χ^2 29.56 with degree of freedom 3. The MAR index ranged from 0.8-1 in our study indicating high resistance among the bacterial isolates and it may be due to soil contamination in and around the pharmaceutical waste site. Other studies from different regions of the globe also suggest that MAR score of greater than 0.2 implies high use of antibiotics (Abdalla *et al.*, 2021; Krumperman, 1983). One study from Mumbai (India) reported MAR index ranged from 0.2 to 0.87 from *Escherichia coli* isolates in Fresh Fish and Fish Waste in Retail Fish Market (Roopa *et al.*, 2023). The MAR score in our case seems to be very high and the reason could be the use of different antibiotic classes with more number of antibiotics for computing MAR index by study from Mumbai. The release of antibiotics in the environment may lead to the creation of resistant gene pool and results in transfer of resistant genes among different bacterial genera.

Variations in antimicrobial susceptibility patterns can be attributed to a variety of factors, including differences in antibiotic utilization practices, the adoption of different infection control techniques, and the availability of public health infrastructure. The need for cautious antibiotic selection in clinical practice to guarantee efficient treatment of bacterial infections and prevent the emergence of antibiotic-resistant strains is further suggested by the differing rates of resistance among antibiotics. The presence of ESBL producers among environmental isolates needs further research to determine whether there is a connection between environmental pollution and the spread of antibiotic-resistance genes among humans and the environment. This work makes a substantial contribution to our knowledge of the environmental reservoir of bacteria that produce ESBLs, especially in pharmaceutical waste dumping locations. The detection of ESBL production among identified bacterial genera highlights emergence of antimicrobial resistance in environmental settings.

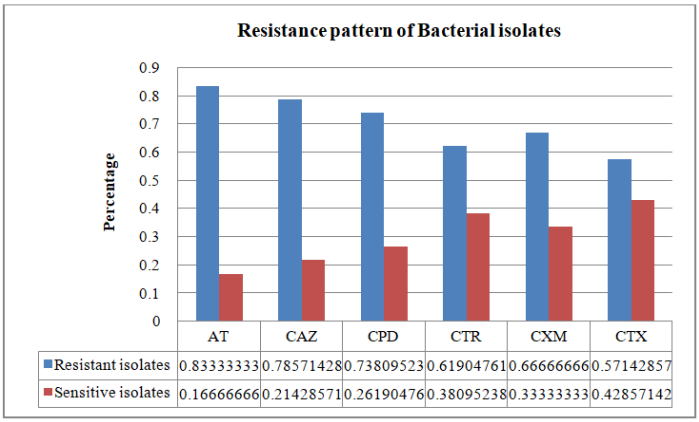

Fig. 1. Resistance/Susceptibility pattern of bacterial isolates against 6 antibiotics tested.

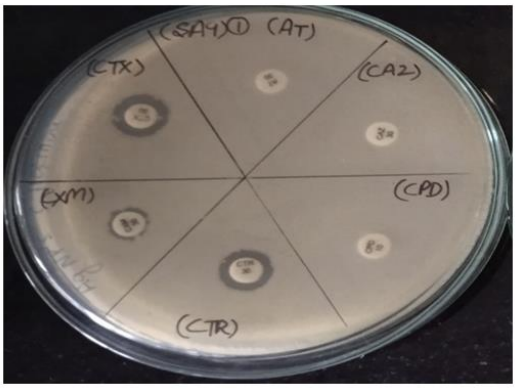

Fig. 2. Isolate showing resistance to all the antibiotics tested.

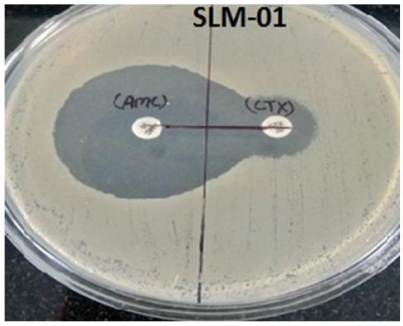

Fig. 3. Isolate no. SLM-01 showing potentiation towards amoxyclav disc in DDST test.

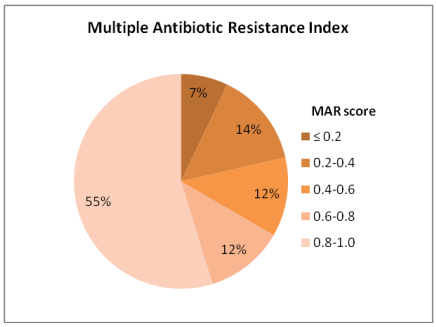

Fig. 4. MAR index showing maximum number of isolates with MAR score between 0.8-1.0.

Table 1: Table of observed values for calculating chi square (χ^2).

Table of Observed Values (Chi-square)								
	MAR score	**1**	**Between 1 - 0.8**	**Between 0.8 - 0.6**	**Between 0.6 - 0.4**	**Between 0.4 - 0.2**	**≤ 0.2**	**Total**
Dataset (all 42 isolates included)	**ESBL phenotype**	16	1	0	0	0	0	17
	Non-ESBL phenotype	0	6	5	5	6	3	25
	Total	**16**	**7**	**5**	**5**	**6**	**3**	**42**
Dataset (only 33 isolates included)	**ESBL phenotype**	16	1	0	0	NA	NA	17
	Non-ESBL phenotype	0	6	5	5	NA	NA	16
	Total	**16**	**7**	**5**	**5**	NA	NA	**33**

Table 2: Antimicrobial susceptibility pattern of different bacterial isolates against antibiotics tested.

Genus	**Antibiotics tested**					
	AT	**CAZ**	**CPD**	**CTR**	**CXM**	**CTX**
Acinetobacter (n=3)	3(100%)	3(100%)	3(100%)	1(33%)	3(100%)	3(100%)
Bacilli (n=3)	3(100%)	3(100%)	3(100%)	3(100%)	2(66%)	1(33%)
Citrobacter (n=2)	0	0	1(50%)	1(50%)	1(50%)	0
Corynebacterium (n=3)	2(66%)	1(33%)	2(66%)	1(33%)	2(66%)	2(66%)
Escherichia (n=3)	2(66%)	3(100%)	3(100%)	3(100%)	3(100%)	2(66%)
Enterobacter (n=1)	1(100%)	1(100%)	1(100%)	0	1(100%)	0
Enterococci (n=1)	1(100%)	1(100%)	1(100%)	0	0	0
Klebsiella (n=5)	4(80%)	3(60%)	2(40%)	2(40%)	2(40%)	2(40%)
Micrococcus (n=1)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)
Proteus (n=2)	2(100%)	2(100%)	1(50%)	1(50%)	1(50%)	1(50%)
Pseudomonas (n=4)	3(75%)	3(75%)	3(75%)	3(75%)	4(100%)	4(100%)
Salmonella (n=3)	3(100%)	3(100%)	3(100%)	3(100%)	3(100%)	2(66%)
Staphylococcus (n=5)	5(100%)	5(100%)	3(60%)	1(20%)	0	1(20%)
Shigella (n=2)	1(50%)	1(50%)	2(100%)	2(100%)	1(50%)	1(50%)
Streptococcus (n=1)	1(100%)	1(100%)	0	1(100%)	1(100%)	1(100%)
Hafnia(n=2)	2(100%)	1(50%)	1(50%)	2(100%)	2(100%)	2(100%)
Flavobacterium (n=1)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)

AT=aztreonam; CAZ=ceftazidime; CPD=Cefpodoxime; CTR=ceftriaxone; CXM=cefuroxime; CTX=cefotaxime

Table 3: Antibiogram of bacterial isolates (n=42) showing resistance (Red), intermediate (Green) and sensitive (Yellow).

Isolate No	Bacterial Genus	Antibiotics Tested						MAR score
		AT	CAZ	CPD	CTR	CXM	CTX	
ACT-01	*Acinetobacter*	R	R	R	R	R	I	1
ACT-02	*Acinetobacter*	R	R	I	S	R	I	0.833333
ACT-03	*Acinetobacter*	R	R	R	S	R	I	0.833333
BC-01	*Bacilli*	I	R	R	I	I	S	0.833333
BC-02	*Bacilli*	I	R	I	I	R	R	1
BC-03	*Bacilli*	R	R	R	R	S	S	0.666667
CTB-01	*Citrobacter*	S	S	R	R	R	S	0.5
CTB-02	*Citrobacter*	S	S	S	S	S	S	0
CNB-01	*Corynebacterium*	S	R	I	I	R	R	0.833333
CNB-02	*Corynebacterium*	R	S	S	S	I	R	0.5
CNB-03	*Corynebacterium*	R	S	R	S	S	S	0.333333
ES-01	*Escherichia*	R	R	R	R	R	R	1
ES-02	*Escherichia*	S	R	R	R	R	S	0.666667
ES-03	*Escherichia*	R	R	R	R	R	R	1
ETB-01	*Enterobacter*	R	R	I	S	I	S	0.666667
ETC-01	*Enterococci*	R	R	R	S	S	S	0.5
KB-01	*Klebsiella*	R	R	R	R	R	R	1
KB-02	*Klebsiella*	R	S	S	S	S	S	0.166667
KB-03	*Klebsiella*	S	S	S	S	S	S	0
KB-04	*Klebsiella*	R	R	R	R	R	R	1
KB-05	*Klebsiella*	R	R	S	S	S	S	0.333333
MC-01	*Micrococcus*	R	R	R	R	R	R	1
PRT-01	*Proteus*	R	R	S	S	S	S	0.333333
PRT-02	*Proteus*	R	R	R	R	R	R	1
PSM-01	*Pseudomonas*	R	I	R	I	R	R	1
PSM-02	*Pseudomonas*	S	S	S	S	R	R	0.333333
PSM-03	*Pseudomonas*	R	R	R	R	R	R	1
PSM-04	*Pseudomonas*	R	R	R	R	R	R	1
SLM-01	*Salmonella*	R	R	R	R	R	R	1
SLM-02	*Salmonella*	R	R	R	R	R	S	0.833333
SLM-03	*Salmonella*	R	R	R	R	R	R	1
STP-01	*Staphylococcus*	R	R	R	S	S	S	0.5
STP-02	*Staphylococcus*	R	R	S	S	S	S	0.333333
STP-03	*Staphylococcus*	R	R	I	I	S	R	0.833333
STP-04	*Staphylococcus*	R	R	S	S	S	S	0.333333
STP-05	*Staphylococcus*	R	R	R	S	S	S	0.5
SHG-01	*Shigella*	R	R	R	R	R	R	1
SHG-02	*Shigella*	S	S	R	R	S	S	0.666667
STR-01	*Streptococcus*	R	R	S	R	R	R	0.833333
HF-01	*Hafnia*	R	R	R	I	R	I	1
HF-02	*Hafnia*	R	S	S	R	R	R	0.666667
FLV-01	*Flavobacterium*	R	R	R	R	R	R	1

Table 4: Relation between ESBL phenotype and MAR score at different significance levels.

Significance level (p-value)	χ^2 tabular		Significant relation between both variables
	Dof 5	Dof 3	
0.05	11.07	7.81	Yes
0.50	4.351	2.366	Yes
0.90	1.610	0.584	Yes
χ^2 calculated= 38.44, P<0.05, degree of freedom (Dof) =5. χ^2 calculated= 29.56, P<0.05, degree of freedom (Dof =3. χ^2 (calculated) > χ^2 (tabular), hence null hypothesis rejected & alternate hypothesis is accepted.			

CONCLUSIONS

Incidence of antimicrobial resistant strains in pharmaceutical waste dumping sites urges the need of impactful waste disposal policies for Pharmaceutical companies so that release of such notorious superbugs in the environment can be controlled. Cefotaxime can be a drug of choice to treat infections due to these organisms but to comprehend the transmission dynamics and molecular interactions between environmental and clinical isolates there is still a scope of extensive research.

FUTURE SCOPE

Molecular characterization of the ESBL producing isolates can be done to categorize the genes responsible for resistance. Further epidemiological studies are needed to identify the mode of gene transfer among bacterial species and to better understand the potential impact on human and animal health.

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Conflict of Interest. None.

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