

## Prevalence of antibiotic resistant among bacterial isolates from Biomedical waste collected from Tiruchiraplli, Tamilnadu, India

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### Abstract

**Background:** Waste environment acts as a reservoir of antibiotic resistance and a potential source of gene resistance among clinical pathogens. Proper management of potentially infectious hazardous waste is most needed among developing countries to reduce drug resistance. Therefore, the present study was aimed to detect the prevalence of antibiotic resistant microbial flora of disposed biomedical waste around Tiruchirappalli, Tamilnadu region.

**Materials and Methods:** Total viable count was determined by pour plate method followed by dilution and antibacterial activity by stroke method against standard antibiotics.

**Results:** A total of 130 samples were processed for the isolation of drug resistance pathogens. Out of 130 samples, 106 samples were positive and about  $272 \times 10^7$  heterotrophic bacterial populations were isolated and identified. Among them the predominant isolates were *E.coli* (25%), *S.aureus* (19.1%) and *P.aeruginosa* (14.3%). Resistant against standard antibiotic revealed that except *S.typhi*, *S.pneumoniae* and *S.pyogens* all other isolated strains showed a high degree of resistance and were multidrug resistant in nature. Among the tested bacterial isolates, *S.aureus*, *P.aeruginosa*, and *E.coli* were found to be multidrug resistance in nature. The maximum MAR index was registered against *P.aeruginosa*, *K.pneumoniae*, *E.coli* and *S.aureus*. The present study concludes

**Conclusion:** This study indicates that high frequency of antibiotic resistance occurred frequently in Gram negative bacteria than Gram positive.

**Keywords:** Biomedical; Heterotrophic; Rifampicin; Amikacin

### Introduction

At present, serious infection caused by microorganism has become difficult to treat because of their resistant to vast array of antibiotics [1]. Antibiotic resistance is the reduction of effectiveness of a drug when it is not intended to kill or inhibit a pathogen due to altered enzyme targets [2]. *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus*, and *Mycobacterium tuberculosis* are most frequently reported as multi drug resistance producers among the hospital acquired and community infections [3]. The volume of antibiotics used in hospitals and released into effluent and municipal sewage provide a selective pressure on bacteria [4,5].

Hospital waste include effluent contain multidrug resistant (MDR) Enterobacteria and enteric pathogens which could arise serious infectious problems. At present the antibiotic resistance has become a major problem in the clinical and public health prospects. The primary pathway for proliferation of antibiotic-resistant bacteria in the environment is mainly in appropriated land filling and administration of antibiotic [6]. The main risk for public health is the resistance genes which are transferred from environmental bacteria to human pathogens. Spread of antibiotic resistance is contributed by indiscriminate and widespread usage of antibiotics. Resistance is a pandemic [7] and complex have a wide range of physiological and biochemical mechanism. The molecular mechanism of drug resistance is a complex and diverse among the microorganisms followed by nature and anthropogenic activities [8]. Furthermore, research on antibiotic resistance reveals that the genetic mutation is a major global factor for the evolution of antibiotic resistance by enzyme inactivation, efflux pump inhibition, altering active site etc. [9,10].

Biomedical waste (BMW) is generated by hospitals, pathological lab, teaching and research institutes, blood banks and animal house. Hospital waste management is an important and mandatory hygiene maintenance system followed by most of the modern hospitals but not strictly by smaller hospitals and nursing homes. Hospital wastes are potential health hazard to the public, flora and fauna of the ecosystem. It increases the pollution of air, land and water has compelled the serious threat of transmission of

disease. Hospital wastes contain both medical and nonmedical wastages may or may not infectious [11]. Infectious waste is higher chance to spread infection to healthy individual and bio toxic to the environment even at low concentration [12]. About 75–90 % of biomedical waste are nonhazardous and 10–25% is hazardous can cause infection. Mismanagement of biomedical waste contains virulent pathogenic bacteria's harbors antibiotic resistant gene by horizontal gene transfer mechanism. Otherwise, high concentration of chemical waste includes antibiotics act as key factor and induces the microbes to adopt the environment by mutation. Among the antibiotic resistance, Beta-lactam antimicrobial agents exhibit the most common bacterial infections and continue to be the prominent cause of resistance to  $\beta$ -lactam antibiotics among Gram negative bacteria worldwide. Mutation of  $\beta$ -lactamases results bacteria expanding their activity even against the newly developed  $\beta$ -lactam antibiotics.

These enzymes are known as Extended-Spectrum  $\beta$ -lactamases [13,14]. Based on protein homology Beta-lactamases are divided in to class A, C, D (serine  $\beta$ -lactamase) and class B enzymes (metallo- $\beta$ -lactamases) [15]. Most ESBLs hydrolyze third-generation cephalosporins although they are inhibited by Clavulanate, and recommend initial screening [16] with either 8 mg/L or 1 mg/L of cefpodoxime, followed by confirmatory tests with both Cefotaxime and Ceftazidime in infection in recent years urges the study to screening of environmental reservoir. Most of the ESBLs belong to class A generally inhibited by Clavulanic acid or Tazobactam. This property distinguishes them from AmpC enzymes, which are class C hydrolyze 3<sup>rd</sup> generation Cephalosporins, but are not inhibited by Clavulanic acid or Tazobactam. Studies shows that the microbiological content of hospital waste is comparable both quantitatively and qualitatively to household waste.

## Materials and methods

### Sample collection

Biomedical wastes such as dressings, body fluid containers, bandages were aseptically collected in a sterile plastic bag from Ariyamangalam garbage pile, Tiruchirapalli, Tamilnadu, India during July 2016 to April 2017.

### Isolation of Pathogens

One g of sample was vigorously mixed with 99 ml normal saline and serially diluted up to  $10^{-9}$ . About one ml of  $10^{-7}$  sample was used for pour plate technique. Blood agar and McConkey agar was used for isolation of pathogen. Colony forming units were counted on nutrient agar plates. Isolates were identified based on grams stain and biochemical characters. The results were expressed as mean  $\pm$  S.D from triplicate dimension [17].

### Antibiotic Susceptibility

Antibiotic susceptibility pattern was performed as described by WHO [18]. The following third generation antibiotics Amikacin, Augmentin, Amoxicillin, Tetracycline, Penicillin, Carbecinillin, Ciproflaxcin, Rifampicin and Cefotaxime were used against the isolated pathogens. After incubation, the results were recorded according to the standard of the Clinical Laboratory Standard Institute (CLSI). Multiple antibiotic resistance (MAR) index was determined for resistant isolate by using the formula MAR = a/b,

a = number of antibiotics to which the test isolate depicted resistance

b = the total number of antibiotics to which the test isolate has been evaluated for susceptibility

## Results and Discussion

### Frequency of Bacterial Strains from Biomedical Waste

This study was attempted to find out the frequency of drug resistance isolates among biomedical waste. A total of 130 waste samples were collected from the mound of garbage pile at 15 different sites of Trichirappalli, Tamilnadu, India. Each site was sampled 3 times with 30 days interval. Totally 130 samples were collected from 45 different sites. From the 130 samples,  $272 \pm 20.9$  bacterial strains belong to ten different genera were isolated. Out of which 100 were Grams positive and 172 were Grams negative (Table 1). The highest number of bacteria was isolated from dressing samples followed by Tissues and body fluids and Bandages. Biochemical characterization of all isolates were performed and identified as *E.coli*, *P.aeruginosa*, *K.pneumoniae*, *P.mirabilis*, *P.vulgaris*, *S.typhi*, *S.aureus*, *Bacillus sp*, *S.pyogens* and *A.baumannii*. The highest prevalence is Grams negative with 63% and Grams positive was 37 %. Of these isolates, *E.coli* (25 %) and *S.aureus* (24.2 %) were most frequently isolated from the samples followed by *P.aeruginosa* (14.3 %) and *K.pneumoniae* (9.1 %). The less frequently isolated strains were *Bacillus sp*, *P.vulgaris* and *S.typhi* (Table 2).

Sample	Sample numbers	Positive samples	Average no of C.F.U	Total isolates (mean $\pm$ SD; n=3)	G <sup>+</sup>	G <sup>-</sup>
Dressings	40	34	$3 \times 10^7$	$120 \pm 1.34$	40	80
Bandages	60	46	$2 \times 10^7$	$80 \pm 1.4$	32	48
Tissues and body fluids	30	26	$2.4 \times 10^7$	$72 \pm 1.8$	28	44
Total	130	106	$7.4 \times 10^7$	272	100	172

Table 1: Total number of isolated bacterial strains and its mean value

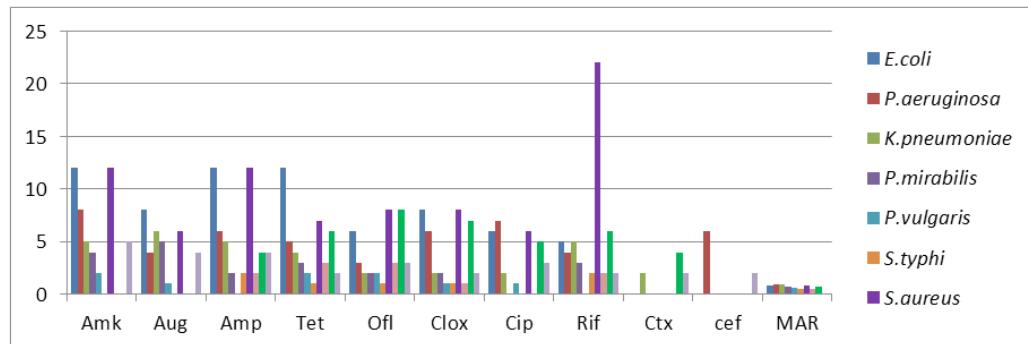
Name of isolates	Dressings	Bandages	Tissues and body fluids	Total		
				n	SD±	Percentage
<i>E.coli</i>	28	26	14	68	6.18	25
<i>P.aeruginosa</i>	22	12	5	39	6.9	14.3
<i>K.pneumoniae</i>	16	8	1	25	6.1	9.1
<i>P.mirabilis</i>	12	2	10	24	4.3	8.8
<i>P.vulgaris</i>	2	0	6	8	2.4	2.9
<i>S.typhi</i>	0	0	8	8	3.7	2.9
<i>S.aureus</i>	26	18	8	52	7.3	19.1
<i>S.pneumoniae</i>	6	6	0	12	2.8	4.4
<i>S.pyogens</i>	2	4	16	22	6.1	8.0
<i>A. baumannii</i>	6	4	4	14	0.9	5.1
Grand total	120	80	72	272	20.9	99.6

**Table 2:** Numbers and Percentage of indigenous genera of isolated strains

### Antibiotic Susceptibility and Resistance Pattern of Isolates

Standard antimicrobial susceptibility of all isolates was given in Table 3. Figure 1 reveals the percentage of antibiotic resistance of isolated bacterial strains. Isolates belongs to *E.coli* and *S.aureus* showed high degree of resistance to all tested antibiotics. Both the strains were found to be resistant against first (pen) and third generation (ctx) antibiotics. Except *P.vulgaris* all other genera extend their resistance against Penicillin, a first generation antibiotic. All the isolates of *P.vulgaris* were 100% sensitive to Ampicillin, Penicillin and Rifampicin. Besides, the third generation antibiotic also did not showed any resistance pattern on *S.typhi* and *Bacillus* sp. Similarly isolates of *S.typhi* and *Bacillus* sp were 100% sensitive to Amikacin, Augmentin, Ciprofloxacin and Cefotaxime. All the genera of isolates except *S.pyogens*, *S.typhi* and *Bacillus* sp were also documented as ESBL producers. Nearly 14 isolates of *E.coli* were detected as resistant to augmentin denotes the extended spectrum of beta lactamase producer. Figure 2 reveals one way ANNOVA analysis of antibiotic resistant. The p-value is .000316 and the result is significant at  $p < .05$ . The data of present study surmises the anthropogenic effect on antibiotic resistance. Disposal of biomedical waste by human beings even the band aid from houses also play significant role in drug resistance developments.

Group	Name of isolates	Amk	Aug	Amp	Tet	Ofl	Clox	Cip	Rif	Ctx	Cef	Mean	MAR
1	<i>E.coli</i>	12	8	12	12	6	8	6	5	0	0	6.9	0.8
2	<i>P.aeruginosa</i>	8	4	6	5	3	6	7	4	0	6	4.9	0.9
3	<i>K.pneumoniae</i>	5	6	5	4	2	2	2	5	2	0	3.3	0.9
4	<i>P.mirabilis</i>	4	5	2	3	2	2	0	3	0	0	2.1	0.7
5	<i>P.vulgaris</i>	2	1	0	2	2	1	1	0	0	0	0.9	0.6
6	<i>S.typhi</i>	0	0	2	1	1	1	0	2	0	0	0.7	0.5
7	<i>S.aureus</i>	12	6	12	7	8	8	6	22	0	0	8.1	0.8
8	<i>S.pneumoniae</i>	0	0	2	3	3	1	0	2	0	0	1.1	0.5
9	<i>S.pyogens</i>	0	0	4	6	8	7	5	6	4	0	4	0.7
10	<i>A. baumannii</i>	5	4	4	2	3	2	3	2	2	2	2.9	0
	Total	48	34	49	45	39	38	34	51	38	26	-	-

**Table 3:** Number of Antibiotic resistance detected from the tested bacterial isolates

Amk: Amikacin, Au: Augmentin, Amp: ampicilin, Tet: Tetracyclin, Ofl: Ofloxacin, Clox: Cloxacilin, Cip: Ciprofloxacin, Rif: Rifampicin, Ctx: Cefotaxime, Cef: Cefepime  
**Figure 1:** Number of antibiotic resistant registered among bacterial isolates

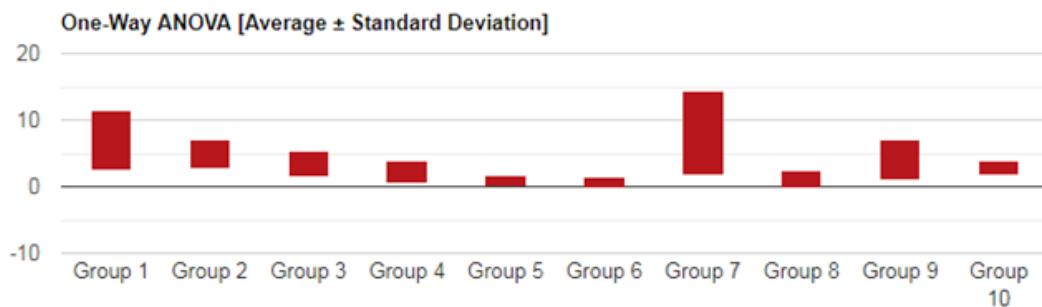


Figure 2: One way ANOVA of antibiotic resistant Isolates

The sampling site is 45 acres which contains wide range of waste material includes polythene bags, bottles, leathers, cosmetics, tablets, house hold wastage etc., a little effort was taken to screen impact of human activities at Aryamangalam garbage mound and antibiotic resistance. The frequency of occurrence of biomedical waste in this mound is very high. Comparing total viable bacterial counts recorded for the three different samples, dressings wastages shows occurrence of microbial population higher than others. The most repeatedly isolated species was *S.aureus* followed by *E.coli* and *P.aeruginosa*, which came in the first place being a principal bacterial isolate among wound infections. Most studies shown that waste effluents from hospitals contain higher levels of antibiotic-resistant and *Acinetobacter* spp. were reported as the target group of bacteria due to their ubiquitous distribution in the environment [19]. The adverse development of antibiotic resistant pattern of *E.coli* as well as other microorganisms due to transfer resistant genes from highly infectious pathogens present in the waste [20]. Similarly *Islam, et al.* [21] reported that isolates of *E. coli* from the liquid hospital waste water rapidly showed resistant to more than 3 kinds of antibiotics. Results indicated that the predominance of ampicillin and rifampicin resistant strains over bacterial representatives. The MAR index of the strains indirectly concludes that all the strains have antibiotic resistance gene originated from the environment and horizontally genetically transferred [22].

## Conclusion

The study was conducted to screen the antibiotic resistant bacteria from biomedical waste disposal. Prevalence of antibiotic resistance was also detected at significant level due to the disposal of biomedical wastes in the environment. This study concludes that multiple antibiotic resistant among *Ecoli*, *P. aeruginosa* and *S.auereus* emerges in the hospital environment.

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