ISSN: 2456-2912
VET 2024; SP-9(3): 97-101

www.veterinarypaper.com
Received: 10-03-2024
Accepted: 12-04-2024

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The study of bacterial flora and their antibiogram pattern isolated from biomedical waste

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Abstract
Biomedical waste is not only a reservoir of microbes but also a potential cause of many new multi drug resistant bacteria. Improper disposal of hospital waste, along with exposure to such waste, poses significant risks to both the environment and human health. In present study, 150 biomedical waste samples were collected different sites such as healthcare facilities, veterinary hospitals and laboratories of Bikaner from Bikaner and all the biomedical waste samples were evaluated for isolation and identification of some aerobic gram- positive and gram-negative bacteria and their antibiotic sensitivity pattern. In the present study, out of 150 biomedical waste samples, 58 (38.66%), 46 (30.66%), 37 (24.66%), 26 (17.33%), 11 (7.33%) were found positive for *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp.* and *Bacillus cereus,* respectively. The antibiotic sensitivity pattern of *E. coli, Pseudomonas aeruginosa, Streptococcus* spp. and *Bacillus cereus* revealed that most effective antibiotic was Methicillin (100%) followed by Azithromycin (73.48%), gentamicin (72.72%), Chloramphenicol (66.66%) and Trimethoprim (65.15%) while the isolates showed high resistance to Clindamycin (78.03%) followed by Ampicillin (71.21%), Penicillin-G(69.69%), Erythromycin (68.93%) and Oxytetracycline (62.12%).The antibiotic sensitivity pattern of *Staphylococcus aureus* revealed that most effective antibiotic was Gentamicin (73.91%) followed by Trimethoprim and Chloramphenicol (69.56%), Ciprofloxacin (63.04%), and Methicillin (45.65%) while the isolates showed highest resistance to Penicillin-G(73.91%) followed by Ampicillin (69.56%), Co-trimoxazole (67.39%), Erythromycin(65.21%), Azithromycin (54.34%), Clindamycin(47.82%) and Methicillin (36.95%). It was concluded that biomedical waste is a major source of multidrug resistant bacteria. Hence, proper management of potentially infectious biomedical waste is needed before disposal.

Keywords: Biomedical waste, bacterial flora, antibiotic sensitivity

Introduction
Biomedical waste, alternatively referred to as infectious medical waste, includes waste produced during activities such as diagnosis, testing, treatment, research, or the manufacture of biological products for human or animal use (Pasupathi *et al*., 2011) [21]. Hospital waste comprises a diverse range of items such as syringes, scalpels, surgical materials like cotton and gloves, bandages, clothing, unused medications, body fluids, tissues, organs, and chemicals (Radha *et al*., 2009) [23]. Healthcare waste includes both organic and inorganic materials that promote the proliferation of harmful microorganisms (Radhakrishna and Nagarajan, 2015) [24]. Approximately 85% of the total waste generated from medical activities is a nonhazardous waste. The remaining 15% is considered potentially hazardous waste (Reddy *et al*., 2023) [25]. Common pathogenic bacteria in bio-medical waste are of the genus *Staphylococci*, *Bacillus* and *Streptococci*, along with varying numbers of other common nosocomial pathogenic bacteria such as *Klebsiella*, *Salmonella*, *Proteus and Enterobacter* species (Alagoz and Kocasoy 2008 and Coker *et al*., 2009) [2, 10]. The primary concern regarding hospital waste and its impact on public health lies in the potential transmission of resistance genes from environmental bacteria to human pathogens. The spread of antibiotic-resistant bacteria in disposed waste poses a significant threat to public health, adversely affecting the population of such environments. (Andy and Okpo, 2018) [4]. Multi-drug resistant pathogens have the capacity to spread not only within local regions but also across the globe. Newly introduced pathogens can spread quickly among vulnerable individuals, particularly those who are immunocompromised (Temitope *et al*., 2016) [27].

Materials and Methods
Sample collection
A total of 150 biomedical waste samples were collected from different sites such as healthcare facilities, veterinary hospitals and laboratories of Bikaner. These samples were placed in sterilized colour coded biohazard bags according to the biomedical waste management rules, 2016 and amendment rules, 2018.

Isolation and identification of bacteria and preservation of pure culture
(A) Preparation and inoculation of samples

Biomedical waste specimens were subjected to elution via the dip method, involving immersing 10 gm of each sample in 90 ml of PBS and shaking for 15 minutes. To confirm microbial dissolution and even distribution in sterile water suspension, each sample was mixed with Nutrient broth in a test tube and then incubated at 37 °C for 24 hours. The resulting inoculum was streaked onto Nutrient agar plates and incubated at 37 °C for 24 hours to observe bacterial proliferation (Anitha *et al*., 2012; Mehara *et al*., 2023) [5, 17].

(B) Identification and biochemical characterisation of isolates

The isolates from biomedical waste samples were identified by analysing their cultural, morphological and biochemical characteristics after incubating them on both Nutrient agar and MacConkey agar. The biomedical waste samples were subjected to aerobic cultivation by streaking each sample on Nutrient agar and MacConkey agar plates in primary, secondary, and tertiary patterns to isolate bacterial colonies. These plates were then incubated at 37 °C for 24 hours. Following incubation, isolated colonies were cultured on Mannitol Salt agar (MSA), Eosine Methylene Blue agar (EMB), Cetrimide agar, Edward's medium base, and Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PEMBA)to isolate *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas* spp., *Streptococcus* and *Bacillus cereus*, respectively. These are commonly found in biomedical waste and can be potentially pathogenic. After another 24 hours of incubation at 37 °C, growth was examined for colony morphology and pigmentation. Once pure colonies were obtained and important features were recorded, further identification was carried out using Gram staining and standard biochemical test kits.

Antibiotic sensitivity pattern of isolates
The method described by Bauer *et al*. (1966) [8] was used to determine the antibiogram of the isolates against different antibiotics. The method involved using 12 various antibiotics. Fresh broth cultures were spread on Mueller-Hinton agar and dried for five minutes. Then antibiotic discs was placed on Muller-Hinton agar. After 12-24 hours of incubation, the inhibition zones were assessed and categorized as either sensitive, intermediate or resistant.

Results and Discussion
In the present study, out of 150 biomedical waste samples 58 (38.66%), 46 (30.66%), 37 (24.66%), 26 (17.33%), 11 (7.33%) samples were found positive for *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp. and *Bacillus cereus*, respectively (Figure1 and Table 1)

Table1: Bacterial species isolated from biomedical waste samples:

S. No.	Bacterial species isolated from biomedical waste samples	No of bacterial species isolated from biomedical waste samples
1	*E. coli*	58 (38.66%)
2	*Staphylococcus aureus*	46 (30.66%)
3	*Pseudomonas aeruginosa*	37 (24.66%)
4	*Streptococcus* spp.	26 (17.33%)
5	*Bacillus cereus*	11 (7.33%)

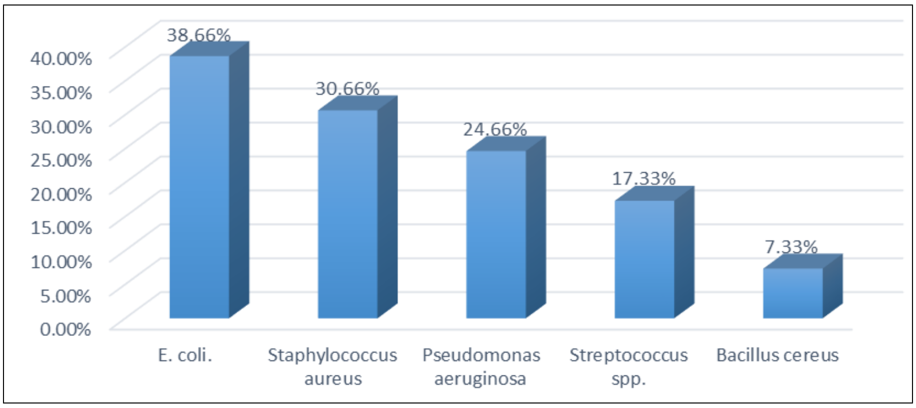

Fig. 1: Percentage of bacterial species isolated from biomedical waste samples

Osagie *et al*. (2016) [20] reported a high prevalence of *Escherichia coli* (39%) and *S. aureus* (32%) which is almost similar to present investigation. The present result corroborates well with the finding of Joshi *et al*. (2020) [12] who isolated aerobic bacteria *viz*. *Escherichia coli* (32%), *Staphylococcus aureus* (28%) and *Bacillus* spp. (10%). Similarly, Omoni *et al*. (2015) [19] screened hospital waste samples and isolated *E. coli* 23(18.6%), *Staphylococcus aureus* (16.1%), *Enterobacter* spp. (14.5%), *Pseudomonas* spp. (12%), *Proteus* spp. (9.7%), *Shigella* spp. (8.9%), *Klebsiella* spp. (6.5%), *Salmonella* spp. (5.7%), *Bacillus* spp. (4.0%), *Citrobacter* spp. (2.4%), and *Serratia* spp. (1.6%). Alwabr *et al*. (2016) [3] isolated bacteria and fungi from hospital solid waste *viz*. *Klebsiella* spp. (9.3%), *E. coli* (12.7%), *Citrobacter* spp. (8.5%), *Candida* spp. (18.6%), *Proteus* spp. (9.3%), *Cladosporium werneckii* spp. (19.5%), *Bacillus* spp. (9.3%), *Aspergillus* spp. (7.6%), *Trichothecium* spp. (0.8%), *Mucor* spp. (3.4%), and *Acinetobacter* spp.

(0.8%). Asfaw *et al*. (2017) [7] investigated 40 hospital waste samples and reported that *Klebsiella* spp. (16.7%)was commonly found in untreated waste followed by *S. aureus* (15.5%) and *Pseudomonas aeruginosa* (14.3%).Prakasam *et al*. (2017) [22] investigated biomedical waste and reported high prevalence of *E. coli* (18.6%), *Enterobacter* spp. (14.5%), *Shigella* spp. (8.9%), *Proteus* spp. (9.7%), *Pseudomonas* spp. (12%), *Serratia* spp. (1.6%), *Staphylococcus aureus* (16.1%), *Klebsiella* (6.5%), *Citrobacter* spp. (2.4%), *Bacillus* spp. (4.0%) and *Salmonella* spp. (5.7%). Hasan *et al*. (2020) [13] evaluated 20 randomly collected samples and isolated *E. coli* (29%), *Pseudomonas* spp. (21.8%), *Klebsiella* spp. (16.4%), *Salmonella* spp. (14.5%), *Staphylococcus* spp (9%). and *Vibrio* spp. (9%).

Antibiotic sensitivity patterns of *E coli, Pseudomonas aeruginosa, Streptococcus* spp. and *Bacillus cereus* isolated from biomedical waste

A total of 132 bacterial isolates included *E. coli*. (58), *Pseudomonas aeruginosa* (37), *Streptococcus* spp. (26) and *Bacillus cereus* (11) isolated from biomedical waste samples were subjected to antibiotic sensitivity test. The antibiotic sensivity pattern revealed that most effective antibiotic was methicillin (100%) followed by azithromycin (73.48%), gentamycin (72.72%), chloramphenicol (66.66%), trimethoprim (65.15%), ciprofloxacin (56.06%) and co-trimoxazole (53.78%) while the isolates showed high resistance to clindamycin (78.03%), followed by ampicillin (71.21%), penicillin-G (69.69%), erythromycin (68.93%) and oxytetracycline (62.12%) as presented in Table 2, Figure 2 and Plate 1.

Table 2: Antibiotic sensitivity patterns of *E coli, Pseudomonas aeruginosa, Streptococcus* spp. and *Bacillus cereus* isolated from biomedical waste

S. No.	Antibiotics	Resistance	Intermediate	Sensitive
1.	Methicillin	0(0%)	0(0%)	132(100%)
2.	Ampicillin	94(71.21%)	11(8.33%)	27(20.45%)
3.	Penicillin-G	92(69.69%)	12(9.09%)	28(21.21%)
4.	Gentamycin	32(24.24%)	4(3.03%)	96(72.72%)
5.	Co-Trimoxazole	54(40.90%)	7(5.30%)	71(53.78%)
6.	Clindamycin	103(78.03%)	10(7.57%)	26(19.69%)
7.	Erythromycin	91(68.93%)	7(5.30%)	34(25.75%)
8.	Oxytetracyclin	82(62.12%)	12(9.09%)	38(28.78%)
9.	Chloroamphenicol	31(23.48%)	13(9.84%)	88(66.66%)
10.	Ciprofloxacin	48(36.36%)	10(7.57%)	74(56.06%)
11.	Trimethoprim	38(28.78%)	8(6.06%)	86(65.15%)
12.	Azithromycin	31(23.48%)	4(3.03%)	97(73.48%)

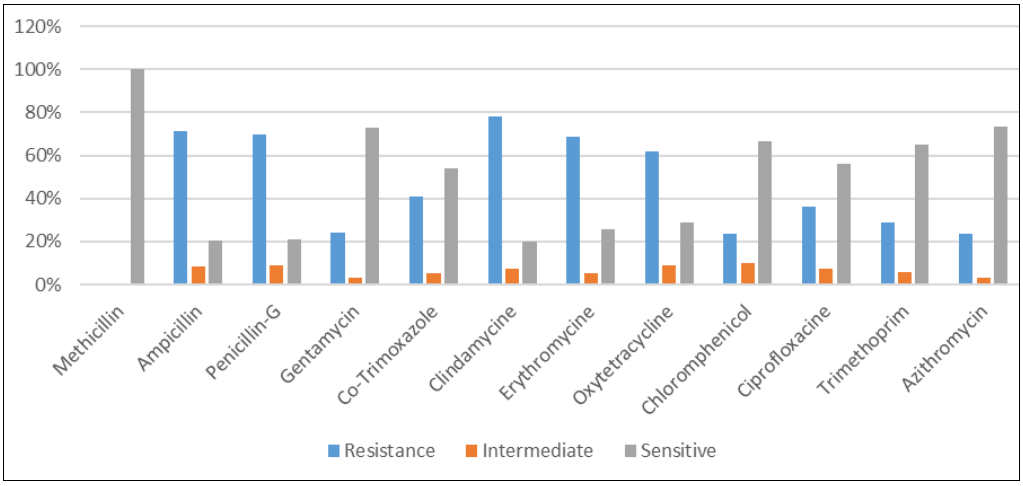

Fig. 2: Antibiotic sensitivity patterns of *E. coli, Pseudomonas aeruginosa, Streptococcus* spp. and *Bacillus cereus*

Plate 1: Antibiotic sensitivity pattern of *E. coli, Pseudomonas aeruginosa, Streptococcus* spp. and *Bacillus cereus*

The present result corroborates well with the finding of Islam *et al*. (2008) [14] found that *E. coli* isolates were resistant to penicillin and erythromycin. Yismaw *et al*. (2010) [28] observed that bacterial isolates were displayed an intermediate level of resistance (60-80%) to ampicillin. However, resistance levels were lower (<60%) to penicillin and erythromycin. Ashfaq*et al*. (2013) [6] showed that the percentage of isolates resistant to various antibiotics including ciprofloxacin, gentamicin and azithromycin were, 27.6%, 24.14% and 13.8%, respectively. Usha *et al*. (2013) [26] showed that bacterial strains were resistant to co-trimoxazole (45.45%).

Antibiotic sensitivity patterns of *Staphylococcus aureus*

A total of 46 *Staphylococcus aureus* isolates recovered from biomedical waste samples were subjected to antibiotic sensitivity test. The antibiotic sensitivity pattern revealed that most effective antibiotic was gentamycin (73.91%), followed by oxytetracycline (71.73%), Trimethoprim and chloramphenicol (69.56%), ciprofloxacin (63.04%), and methicillin (45.65%) while the isolates showed highest resistance to penicillin-G (73.91%) followed by ampicillin (69.56%), co-trimoxazole (67.39%), erythromycin (65.21%), azithromycin (54.34%) and clindamycin (47.82%) as presented in Table 3, Figure 3 and Plate 2.

Table 3: Antibiotic sensitivity patterns of *Staphylococcus aureus*:

S. No.	Antibiotics	Resistance	Intermediate	Sensitive
1.	Methicillin	17(36.95%)	8(17.39%)	21(45.65%)
2.	Ampicillin	32(69.56%)	7(15.21%)	7(15.21%)
3.	Penicillin-G	34(73.91%)	5(10.86%)	7(15.21%)
4.	Gentamycin	8(17.39%)	4(8.69%)	34(73.91%)
5.	Co-Trimoxazole	31(67.39%)	2(4.34%)	13(28.26%)
6.	Clindamycin	22(47.82%)	3(6.52%)	21(45.65%)
7.	Erythromycin	30(65.21%)	5(10.86%)	11(23.91%)
8.	Oxytetracycline	9(19.56%)	4(8.69%)	33(71.73%)
9.	Chloromphenicol	11(23.91%)	3(6.52%)	32(69.56%)
10.	Ciprofloxacin	15(32.60%)	2(4.34%)	29(63.04%)
11.	Trimethoprim	11(23.91%)	3(6.52%)	32(69.56%)
12.	Azithromycin	25(54.34%)	4(8.69%)	17(36.95%)

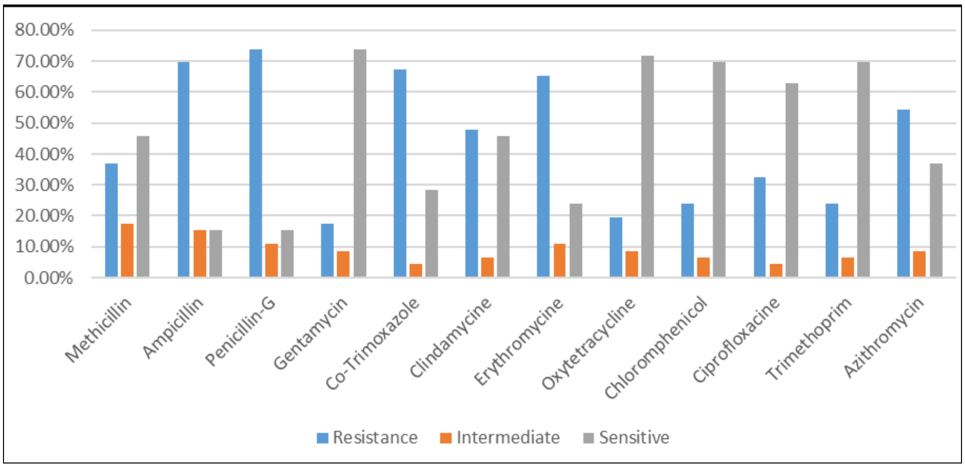

Fig 3: Antibiotic sensitivity patterns of *Staphylococcus aureus*

Kalantar *et al*. (2008) [15] found that *Staphylococcus aureus isolates* were resistant to co-Trimoxazole and ampicillin. Kumari *et al*. (2008) [16] observed that *Staphylococcus aureus* strains were resistant to penicillin and erythromycin. Olowe *et al*. (2013) [18] reported that the *S. aureus* isolates were resistant to penicillin (82.7%), but susceptible to gentamicin (88.5%). Adetayo *et al*. (2014) [1] reported that *Staphylococcus aureus* isolates were showed 30.4% resistance for methicillin, 71.4% for Cotrimoxazole and 42.9% for gentamycin.

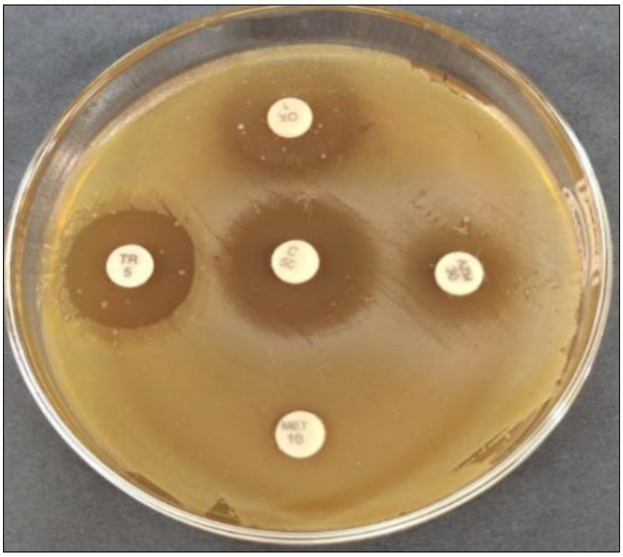

Plate 2: Antibiotic sensitivity pattern of *Staphylococcus aureus*

Conclusion
The present study contributed to better understanding of the bacterial flora and their antibiotic resistant pattern isolated biomedical waste. The antibiotic resistance pattern of bacterial isolates differs and depending on the specific geographic area and the antibiotics commonly used there. Furthermore, the isolation of multidrug resistant bacteria in biomedical waste samples indicated the potential public health hazard due to improper disposal of hospital waste. Hence, proper management of potentially infectious biomedical waste is needed before disposal.

Conflict of Interest
Not available

Financial Support
Not available

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How to Cite This Article
Nikita, Sain ML, Goklaney D, Vaishali CD. The study of bacterial flora and their antibiogram pattern isolated from biomedical waste. International Journal of Veterinary Sciences and Animal Husbandry. 2024; SP-9(3): 97-101.