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Bacterial isolates and their antibiogram from biomedical waste

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Abstract
A study was conducted on 90 biomedical waste samples obtained from a veterinary clinical complex to analyze the presence of bacterial species, their identification, and antibiogram. A total of 153 bacterial agents were isolated from these samples. Among them, 46 samples (51.11%) tested positive for *E. coli*, 32 samples (35.55%) tested positive for *Staphylococcus aureus*, 29 samples (32.22%) tested positive for *Klebsiella* spp., 20 samples (22.22%) tested positive for *Pseudomonas aeruginosa*, 14 samples (15.55%) tested positive for *Bacillus* spp., and 12 samples (13.33%) tested positive for *Streptococcus* spp. The antimicrobial resistance profile of these bacterial isolates was assessed against various antibiotics. The results revealed that the isolates exhibited sensitivity to gentamicin (90.84%), levofloxacin (88.88%), ciprofloxacin (86.27%), vancomycin (79.73%), co-trimoxazole (sulpha/trimethoprim) (78.43%), streptomycin (76.47%), and chloramphenicol (58.82%). However, the isolates showed resistance to ampicillin (86.27%), clindamycin (85.62%), oxacillin (84.96%), amoxyclav (84.96), erythromycin (80.39%), linezolid (79.73%), penicillin–G (56.20%), tetracycline (53.59%), and sulfatriad (53.59%). All the isolates exhibited resistance to at least five or six drugs, indicating a significant finding. Moreover, the Gram-negative bacteria demonstrated higher resistance to the antibiotics tested as compared to the Gram-positive bacteria.

Keywords: Bacterial isolates, antibiogram, biomedical waste

Introduction
Antibiotics are typically effective in destroying the majority of bacteria in a colony. It is possible, however, that there exists a separate colony of bacteria that has been genetically altered, resulting in resistance. It has been demonstrated that the prevalence of antibiotic-resistant illnesses is significantly correlated with the amount of antibiotics consumed. Throughout the world, antibiotic resistance is becoming an increasing problem due to the longevity of bacterial infections. It is no secret that antibiotic resistance has attracted a great deal of attention from the medical community and researchers over the past few decades. In order to address the prevalence of antibiotic resistance, it is essential that attention be paid to the consequences of resistance spread when treating the disease (Prasad *et al.* 2018) [13].

Biomedical waste generated in health care facilities are at present, collected without segregation into infectious and non-infectious categories and are disposed of in municipal containers located either inside or outside the facility premises. Wastes from operation theatres, hospital wards and pathological laboratories, and research laboratories are disposed of without any disinfection/sterilisation. The major public health threat posed by hospital waste is the transfer of resistance genes from ambient bacteria in the environment to animal or human infections (Pattniak *et al.*, 2013) [12]. Biomedical waste is a breeding ground for various bacteria and serves as a breeding ground for multidrug resistance owing to antibiotics, disinfectants, and metabolised drugs from patients' body fluids, contributing to the high prevalence of drug-resistant bacteria (Anssour *et al.* 2018; Alam and Imran 2018) [2, 1].

Antibiotic-resistant organisms can cause life-threatening infections that are difficult to treat because of limited treatment options. Characterization of bacteria from hospital settings provides information about the prevalence of antibiotic resistance among them and helps prevent the development of resistance to common antibiotics (Fekadu *et al.* 2015). [8] The most serious problem associated with antibiotic-resistant bacteria is the increase in antibiotic resistance among microorganisms that are dangerous to humans and animals, making it difficult to treat some life-threatening diseases (Pruden *et al.*, 2013) [14].

Antibiotic resistance in bacteria has been called a major public health risk. Antibiotic susceptibility testing helps determine and select drugs that are effective against a specific disease caused by a specific bacterium. Choosing the right antibiotic reduces both the cost of therapy and the length of recovery.

Materials and Methods

Collection of samples

A total of 90 samples of biomedical waste were collected from various locations, such as health facilities, veterinary clinics, and clinical laboratories in Bikaner district, and packaged in sterilised, colour-coded Biohazard bags in accordance with the Biomedical Waste Management Rules, 2016 and the Amendment Rules, 2018.

Approximately 100–150 grams of biomedical waste was collected in pre-sterilized, color-coded bags and taken to the laboratory of the Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Science, Bikaner, and Animal Biomedical Waste Disposal Technology Center. Animal or human tissues, body parts, blood, body fluids, infectious animal litter, cotton dressings, syringes with needles, lancets, scalpels, razor blades and precision knives, contaminated glass fragments, blood ampoules, and other items that may cause punctures or cuts were considered and collected as biomedical waste.

At the time of collection, all necessary precautions were followed by wearing protective clothing (apron), gloves, face mask, headgear and rubber boots as prescribed in the Bio-Medical Waste Management Rules, 2016 [5] (Amendment Rules, 2018).

Bacterial isolation, identification and biochemical characterization

(a) Preparation and Inoculation of Samples

Solid biomedical waste samples were brought to elute using the dip method. In this method, 10 grams of each sample was immersed in 90 ml of PBS and the samples were shaken for 15 minutes. To verify that the microorganisms were dissolved and evenly distributed in the suspended sterile water, each waste sample was thoroughly mixed with nutrient broth in a test tube and then incubated at 37ºC for 24 hours. Prepared inoculum was streaked onto the nutrient agar plate and plates incubated at 37ºC for 24 hours to observe bacterial growth.

Isolation of bacteria and preservation of pure cultures

(a) Isolation of Bacteria

After counting the plates, pure cultures of microorganisms were isolated by removing one representative of each colony from the crowded plate with a sterile wire loop. The subcultures were placed on solidified sterile nutrient agar plates for bacterial isolates or spread under aseptic conditions. The plates were incubated upside down at 37°C for 24 hours. The plates were examined for pure isolates; the pure cultures obtained were transferred to separate sterile culture media and stored in the refrigerator as stock cultures, from which routine samples were taken for biochemical tests and microscopy (Fawole and Oso, 2001). [7]

Characterization and identification of bacterial isolates

Bacterial isolates were identified based on the isolates' colonial morphology and biochemical characteristics.

(a) Bacterial Identification

A total of six bacteria were characterized and identified during the course of the study. The bacteria comprise of both gram positive and gram negative bacteria which include the following: *Staphylococcus aureus, E. coli, Klebsiella* spp., *Pseudomonas aeruginosa, Bacillus cereus and Streptococcus* spp.

(b) Colonial Morphology

The colonial morphology used in the identification of bacterial isolates include the colony colour, shape, pigmentation, optical characteristics and colonial edges which were all observed directly on plates after appropriate incubation.

(c) Antibacterial Sensitivity Test

All 153 isolates associated with biomedical waste were subjected to an *in vitro* antibiotic susceptibility test using the disc diffusion technique (Bauer *et al*., 1966) [4]. Sixteen different antibiotics were commonly used. Fresh broth cultures were spread on Mueller-Hinton agar and impregnated with antibiotic plates. These plates were incubated for 12-24 hours and the inhibition zones were measured and classified as sensitive, moderately sensitive and resistant according to the manufacturer's table.

Results

The following bacteria were tested for antibiotic sensitivity in the current study: *Staphylococcus aureus, E. coli, Klebsiella* spp., *Pseudomonas aeruginosa, Bacillus,* and *Streptococcu* spp. sixteen different antibiotics were used to treat bacterial isolates for this. Among the antibiotics used were ampicillin, ciprofoxacin, gentamicin, linezolid, streptomycin, vancomycin, oxacillin, erythromycin, tetracycline, chloramphenicol, clindamycin, co-trimoxazole (sulpha/trimethoprim), levofloxacin, penicilline-G, sulphatriad, and amoxyclav.

There were 153 different bacterial isolates in all 90 biomedical waste sample. There were 46 positive samples for *E. coli* (51.11%), 32 positive samples for *Staphylococcus aureus* (35.55%), 29 positive samples for *Klebsiella* spp. (32.22%), 20 positive samples for *Pseudomonas aeruginosa* (22.22%), 14 positive samples for *Bacillus* cereus (15.55%), and 12 positive samples for *Streptococcus* spp. (13.33%).

Because resistant bacteria can adapt to different environmental conditions and act as vectors for the spread of antibiotic resistance genes, antibiotics exert selection pressure in their favor by killing or impeding the growth of susceptible bacteria (Subramaniam, G., and Girish, M. 2020) [15]. The pattern of antibiotic susceptibility for bacteria species isolated from study samples is shown in Table 3. Sixteen (16) antibiotics were used in a susceptibility test on every bacterial species that was isolated from a biomedical waste sample.

Antibiotics have been used as a prophylactic strategy in a range of areas, including animal husbandry and agriculture, for decades (Gajdacs M and Albericio F. 2019) [9]. AMR is the capacity of bacteria and other microbes to resist the effects of an antibiotic to which they were previously susceptible, enabling germs to survive and spread (Zaman *et al*., 2017) [17]. AMR is inevitable because microorganisms produce genetic changes to mitigate its lethal effects (Subramaniam, G., and Girish, M. 2020) [15].

Antibiotic susceptibility test results for all bacterial isolates were interpreted according to the literature provided by the manufacturer. The responses of the organisms to antibiotics were categorized as sensitive, moderately sensitive, and resistant. All isolates showed varying percentages of sensitivity and resistance to all antibiotics used. The antimicrobial resistance profile of the bacterial isolates to different antibiotics showed that the isolates were sensitive to gentamicin (90.84%), levofloxacin (88.88%), ciprofloxacin (86.27%), vancomycin (79.73%), co-trimoxazole (sulpha/trimethoprim) (78.43%), streptomycin (76.47%) and chloramphenicol (58.82%), while isolates were resistant to ampicillin (86.27%), clindamycin (85.62%), oxacillin (84.96%), amoxyclav (84.96), erythromycin (80.39%), linezolid (79.73%), penicillin–G (56.20%), tetracycline (53.59%) and sulfatriad (53.59%). All isolates were resistant to five or more drugs. Gram-negative bacteria were found to be more resistant to the antibiotics tested than Gram-positive bacteria.

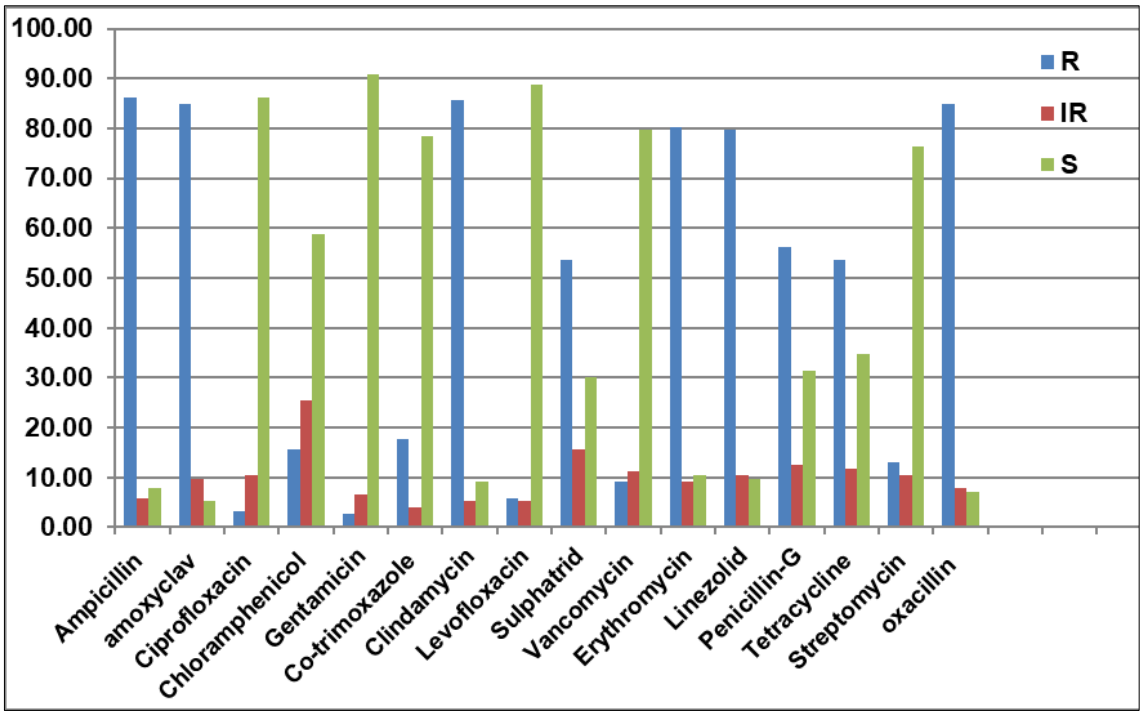

Fig 1: Antibiotic resistance profile of bacteria isolated from biomedical waste samples

Table 1: Antibiotic resistance profile of bacteria isolated from biomedical waste samples

Serial No.	Antibiotic	Resistance	Intermediate resistance	Susceptible
1.	Oxacillin (1 µg)	130(84.96)	12(7.84)	11(7.18)
2.	Erythromycin (15 µg)	123(80.39)	14(9.15)	16(10.45)
3.	Tetracycline (30 µg)	82(53.59)	18(11.76)	53(34.64)
4.	Ampicillin (10/10 µg)	132(86.27)	9(5.88)	12(7.84)
5.	Sulphatriad (300 µg)	82(53.59)	24(15.68)	47(30.71)
6.	Gentamicin (10 µg)	4(2.61)	10(6.53)	139(90.84)
7.	Linezolid (30 µg)	122(79.73)	16(10.45)	15(9.80)
8.	Streptomycin (10 µg)	20(13.70)	16(10.45)	117(76.47)
9.	Vancomycin (30 µg)	14(9.15)	17(11.11)	122(79.73)
10.	Co-Trimoxazole (Sulpha/ Trimethoprim) (25 µg)	27(17.64)	6(3.92)	120(78.43)
11.	Levofloxacin (5 µg)	9(5.88)	8(5.22)	136(88.88)
12.	Clindamycin (2 µg)	131(85.62)	8(5.22)	14(9.15)
13.	Chloramphenicol (30 µg)	24(15.68)	39(25.49)	90(58.82)
14.	Ciprofloxacin (30 µg)	5(3.26)	16(10.45)	132(86.27)
15.	Penicillin -G (10 unit)	86(56.20)	19(12.41)	48(31.37)
16.	Amoxyclav (30 µg)	130(84.96)	15(9.80)	8(5.22)

Discussion

Aziz *et al*. (2014) [3] found that bacterial isolates were resistant to ampicillin (80%), followed by amoxicillin/clavulanate (77%). Omoni *et al*. (2015) [11] found that Gram-negative bacterial isolates had the highest resistance to ampicillin (84%), while they showed the highest sensitivity to gentamicin (77.7%) and streptomycin (77.7%). Gram-positive isolates, on the other hand, showed the highest resistance to erythromycin (64%) and the highest sensitivity to streptomycin (96%) and gentamycin (84%). Mwaikono *et al*. (2015) [10] isolated bacteria that were resistant to penicillin G. ciprofloxacin and gentamycin. Tuem *et al*. (2018) [16] reported that bacterial isolates were resistant to ampicillin (83.81%). Aziz *et al*. (2014) [3]; Omoni *et al*. (2015) [11]; Mwaikono *et al*. (2015) [10] and Tuem *et al*. (2018) [16] are almost similar and corroborates with the present findings. The differences in the antibiogram profile of the bacterial isolates could be due to different bacterial strains and antibiotic doses.

Conclusion

Biomedical waste is a potential source and reservoir of antibiotic-resistant microorganisms. The spread of antibiotic-resistant bacteria in discarded biomedical waste poses a threat to public health and has a negative impact on the local population. Considering the previously discussed impacts, biological waste should be properly treated before being disposed of in the environment and effective waste management techniques should be implemented in our hospitals.

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