



Bacterial isolation, identification, and antimicrobial susceptibility pattern from biomedical waste samples

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Manuscript details:

Received: 26.04.2026

Accepted: 15.06.2026

Published: 30.07.2026

Cite this article as:

Jane Rasika R and Kale ES (2026) Bacterial isolation, identification, and antimicrobial susceptibility pattern from biomedical waste samples. *Int. J. of Life Sciences*, 14 (2): 149-156.

Available online on <http://www.ijlsci.in>

ISSN: 2320-964X (Online)

ISSN: 2320-7817 (Print)



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ABSTRACT

Biomedical waste from healthcare facilities carries a substantial burden of pathogenic microorganisms capable of causing hospital-acquired infections and propagating antimicrobial resistance (AMR). The inadequate disposal of such waste poses a direct threat to healthcare workers, patients, and the surrounding community. This study aimed to isolate and identify bacteria present in biomedical waste samples collected from IRWIN Hospital, Amravati, and to determine the antimicrobial susceptibility patterns of the recovered isolates using standard disc diffusion methodology. Twenty biomedical waste samples (BMW01–BMW20) were collected aseptically from various hospital sites. Bacterial identification was based on colony morphology, Gram staining, motility, and biochemical characterization (IMViC, catalase, urease, and sugar fermentation tests). Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method on Mueller Hinton agar for four antibiotics — Ampicillin (AMP, 10 mcg), Tetracycline (TE, 30 mcg), Gentamicin (GEN, 10 mcg), and Methicillin (MET, 5 mcg) — with interpretation based on CLSI (2025) guidelines. A total of 40 bacterial isolates were recovered. *Escherichia coli* was the most prevalent organism (n=15; 37.5%), followed by *Staphylococcus aureus* (n=12; 30%), *Klebsiella pneumoniae* (n=7; 17.5%), and *Pseudomonas aeruginosa* (n=6; 15%). All isolates showed complete resistance (100%) to Ampicillin. Methicillin resistance was universal except where intrinsic resistance rendered testing irrelevant for Gram-negatives. Multidrug resistance (MDR) was confirmed in all *K. pneumoniae* and *P. aeruginosa* strains. MRSA characteristics were identified in all *S. aureus* isolates. Biomedical waste is a critical reservoir for MDR pathogens. Urgent enforcement of waste management protocols and rational antibiotic stewardship are essential to curb infection risk and AMR dissemination.

Keywords: Biomedical waste, antimicrobial resistance, MRSA, multidrug resistance, Kirby-Bauer, nosocomial infections

INTRODUCTION

Healthcare activities generate a diverse range of waste materials, collectively termed biomedical waste (BMW), which encompasses used syringes, blood-soaked cotton, surgical dressings, expired biological products, sharps, and laboratory specimens. The World Health Organization (WHO) classifies approximately 10–25% of this waste as

hazardous, with the remainder generally non-hazardous though contamination of non-hazardous streams by infectious material elevates the risk profile of the entire waste load (Pasupathi et al., 2011; Hirani et al., 2014).

Improper segregation, handling, and disposal of BMW have well-documented consequences: the spread of pathogens among healthcare workers and the public, soil and water contamination, promotion of antimicrobial resistance through persistent microbial selection pressure, and economic burden from resultant morbidity (Pruess-Ustun et al., 2014). Hospital-acquired infections (HAIs), caused by organisms including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, are frequently linked to biomedical waste as a source of microbial dispersal (Bansod & Deshmukh, 2023; Medubi et al., 2006). Many of these pathogens have developed resistance to multiple antibiotic classes, giving rise to methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, and carbapenem-resistant organisms.

Despite the known public health significance of BMW contamination, microbiological surveillance of improperly discarded hospital waste remains limited, especially in resource-constrained settings. This study was conducted to fill this gap by characterizing the bacterial load and resistance profile of BMW samples collected from IRWIN Hospital, Amravati, Maharashtra.

Aim and Objectives

Aim: To isolate, identify, and determine the antimicrobial susceptibility pattern of bacteria recovered from biomedical waste samples.

Objectives:

- Collect biomedical waste samples from designated hospital sites under aseptic conditions.
- Isolate and culture bacteria using appropriate selective and differential microbiological media.
- Identify isolates on the basis of morphological, cultural, and biochemical characteristics.
- Determine antimicrobial susceptibility patterns using the Kirby-Bauer disc diffusion method.
- Characterize the multidrug resistance (MDR) profile of the recovered organisms.

- Evaluate public health risks associated with pathogenic and antibiotic-resistant bacteria in BMW.
- Highlight the importance of regulated biomedical waste management in preventing infection dissemination.

MATERIALS AND METHODS

Sample Collection

Twenty biomedical waste samples (coded BMW01–BMW20) were collected from identified waste-generating areas within IRWIN Hospital, Amravati — including wards, laboratories, operation theatres, and designated disposal units. Each sample was obtained under aseptic conditions using sterile plastic containers and zip-lock bags. Personal protective equipment (gloves, mask, and apron) was worn throughout the collection process. Samples were properly labelled with date, time, and collection site, transported to the microbiology laboratory, and processed within 24 hours of collection to minimize any viability-related bias (Saini et al., 2004).

Culture Media

All culture media were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. The following selective and differential media were employed: Nutrient Agar (NA) as a primary non-selective medium; MacConkey Agar (MCA) for Gram-negative coliform differentiation; Eosin-Methylene Blue (EMB) Agar for confirmatory identification of *E. coli*; Mannitol Salt Agar (MSA) and Baird-Parker Agar (BPA) for *S. aureus*; Simmon's Citrate Agar (SCA) for *K. pneumoniae*; Cetrimide and Pseudomonas Agar for *P. aeruginosa*; and Mueller Hinton Agar (MHA) for antibiotic susceptibility testing.

Isolation Procedure

Serial dilutions of each sample were prepared in sterile normal saline up to 10^{-5} . Using the pour plate method, 1 mL of the appropriate dilution was inoculated onto Nutrient Agar and incubated at 37°C for 24 hours. Distinct colonies were subsequently sub-cultured on selective and differential media specific to each target organism, as outlined below.

E. coli was identified by characteristic pink colonies on MacConkey agar and metallic green colonies on EMB agar. *P. aeruginosa* produced green-blue colonies on Pseudomonas agar due to pyocyanin pigment

production. *K. pneumoniae* formed large, mucoid, pink colonies on MacConkey agar and showed a positive citrate reaction (green-to-blue colour change) on Simmon's Citrate Agar. *S. aureus* produced golden-yellow colonies on Nutrient Agar, yellow colonies with yellow zones (mannitol fermentation) on MSA, and black colonies on Baird-Parker Agar.

Biochemical Identification

Each isolate was confirmed through a battery of biochemical tests: the IMViC series (Indole production with Kovac's reagent; Methyl Red test; Voges-Proskauer test with alpha-naphthol and KOH; Citrate utilization on Koser's medium), catalase activity (H₂O₂ effervescence on colony), urease production, and sugar fermentation (glucose, lactose, sucrose, mannitol) in Durham tube broths. Gram staining and motility testing were performed for all isolates (Jayraaj et al., 2013; Narayan & Paniker, 2017).

Antibiotic Susceptibility Testing (AST)

AST was performed by the Kirby-Bauer disc diffusion method on Mueller Hinton Agar, following CLSI (2025) guidelines. A bacterial suspension adjusted to a 0.5 McFarland turbidity standard was swabbed uniformly across MHA plates. Antibiotic discs — Ampicillin (AMP, 10 mcg), Tetracycline (TE, 30 mcg), Gentamicin

(GEN, 10 mcg), and Methicillin (MET, 5 mcg) — were placed equidistant on the inoculated plate using sterile forceps. After 30 minutes of pre-diffusion at room temperature, plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimetres and interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to CLSI breakpoints.

RESULT AND DISCUSSION

Sample Collection and Bacterial Isolation

A total of 40 bacterial isolates were recovered from 20 biomedical waste samples. Table 1 lists each sample code, its source, and the organisms isolated.

Distribution of Bacterial Isolates

E. coli was the predominant isolate (n=15; 37.5%), followed by *S. aureus* (n=12; 30%), *K. pneumoniae* (n=7; 17.5%), and *P. aeruginosa* (n=6; 15%). Table 2 presents the distribution across sample sources.

Morphological and Biochemical Characteristics

Organisms were confirmed by Gram staining, motility testing, and biochemical profiling. Table 3 summarizes the results.

Table 1: Biomedical waste samples and corresponding bacterial isolates (IRWIN Hospital, Amravati)

Sample Code	Sample Source	Bacterial Isolates
BMW01	Used Syringe	<i>S. aureus</i> , <i>P. aeruginosa</i>
BMW02	Blood-soaked cotton	<i>S. aureus</i> , <i>K. pneumoniae</i>
BMW03	Used gloves	<i>E. coli</i> , <i>K. pneumoniae</i>
BMW04	IV set tube	<i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i>
BMW05	Urine bag	<i>E. coli</i> , <i>P. aeruginosa</i>
BMW06	Dressing materials	<i>E. coli</i> , <i>S. aureus</i>
BMW07	Used masks	<i>E. coli</i> , <i>K. pneumoniae</i>
BMW08	Waste bin surface	<i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i>
BMW09	Expired blood bag	<i>S. aureus</i> , <i>K. pneumoniae</i>
BMW10	Dialysis tube	<i>E. coli</i> , <i>P. aeruginosa</i>
BMW11	Urine container	<i>E. coli</i> , <i>S. aureus</i>
BMW12	Used EDTA tube	<i>E. coli</i>
BMW13	Pipette tips	<i>E. coli</i> , <i>S. aureus</i>
BMW14	Stool container	<i>S. aureus</i>
BMW15	Used test cards	<i>E. coli</i> , <i>P. aeruginosa</i>
BMW16	Surgical blades	<i>E. coli</i> , <i>S. aureus</i>
BMW17	Spirit swab after injection	<i>E. coli</i> , <i>K. pneumoniae</i>
BMW18	Pus-soaked bandage	<i>E. coli</i> , <i>S. aureus</i>
BMW19	Lancet disposal box	<i>E. coli</i> , <i>K. pneumoniae</i>
BMW20	Glucometer strip discard	<i>S. aureus</i> , <i>K. pneumoniae</i>

Table 2: Distribution of bacterial isolates across biomedical waste sources

Organism	Isolates (n)	Percentage	Sample Sources
<i>Escherichia coli</i>	15	37.5%	Used gloves, IV set, urine bag, dressing materials, masks, waste bin, dialysis tube, urine container, EDTA tube, pipette tips, test cards, blades, spirit swabs, bandage, lancet box
<i>Staphylococcus aureus</i>	12	30.0%	Used syringe, blood cotton, IV set, dressing, waste bin, blood bag, urine container, pipette tips, stool container, blades, bandage, glucometer strips
<i>Klebsiella pneumoniae</i>	7	17.5%	Blood-soaked cotton, used gloves, used masks, expired blood bag, spirit swabs, lancet disposal box, glucometer strips
<i>Pseudomonas aeruginosa</i>	6	15.0%	Used syringe, IV set tube, urine bag, waste bin surface, dialysis tube, used test cards
Total	40	100%	—

Table 3: Morphological and biochemical characteristics of bacterial isolates

Organism	Gram Stain	Motility	Spore	Indole	MR	VP	Citrate	Catalase	Glucose	Lactose	Mannitol	Sucrose	Urease
<i>E. coli</i>	Gram –ve rods	Motile	Non-spore	+	+	–	–	+	+	+	+	+	+
<i>S. aureus</i>	Gram +ve cocci	Non-motile	Non-spore	–	+	+	+	+	+	+	+	+	+
<i>K. pneumoniae</i>	Gram –ve rods	Non-motile	Non-spore	+	–	+	+	+	+	+	+	+	+
<i>P. aeruginosa</i>	Gram –ve rods	Motile	Non-spore	–	–	–	+	+	–	–	–	–	–

MR = Methyl Red; VP = Voges-Proskauer; + = positive reaction; – = negative reaction

Table 4: Zone of inhibition (mm) for *E. coli* isolates (n=15)

Isolate	AMP (mm)	TE (mm)	GEN (mm)	MET (mm)
EC1	13(R)	14(I)	15(S)	0(R)
EC2	11(R)	11(I)	17(S)	0(R)
EC3	10(R)	10(I)	15(S)	0(R)
EC4	13(R)	12(I)	15(S)	0(R)
EC5	12(R)	14(I)	16(S)	0(R)
EC6	9(R)	13(I)	18(S)	0(R)
EC7	11(R)	11(I)	20(S)	0(R)
EC8	8(R)	12(I)	21(S)	0(R)
EC9	5(R)	12(I)	18(S)	0(R)
EC10	2(R)	12(I)	15(S)	0(R)
EC11	13(R)	14(I)	17(S)	0(R)
EC12	10(R)	14(I)	17(S)	0(R)
EC13	12(R)	11(I)	19(S)	0(R)
EC14	13(R)	11(I)	15(S)	0(R)
EC15	13(R)	14(I)	15(S)	0(R)

R = Resistant; I = Intermediate; S = Sensitive (CLSI 2025 breakpoints)

Table 5: Zone of inhibition (mm) for *S. aureus* isolates (n=12)

Isolate	AMP (mm)	TE (mm)	GEN (mm)	MET (mm)
SA1	14(R)	21(S)	11(R)	21(R)
SA2	10(R)	19(S)	8(R)	9(R)
SA3	9(R)	22(S)	10(R)	20(R)
SA4	4(R)	21(S)	7(R)	8(R)
SA5	11(R)	20(S)	11(R)	19(R)
SA6	8(R)	19(S)	9(R)	10(R)
SA7	13(R)	22(S)	5(R)	14(R)
SA8	10(R)	22(S)	7(R)	11(R)
SA9	12(R)	21(S)	4(R)	15(R)
SA10	14(R)	25(S)	8(R)	13(R)
SA11	10(R)	24(S)	12(R)	16(R)
SA12	11(R)	26(S)	2(R)	12(R)

R = Resistant; I = Intermediate; S = Sensitive

Table 6: Zone of inhibition (mm) for *K. pneumoniae* (KPN) and *P. aeruginosa* (PA) isolates

Isolate	AMP (mm)	TE (mm)	GEN (mm)	MET (mm)
KPN1	0(R)	11(R)	12(R)	0(R)
KPN2	0(R)	8(R)	10(R)	0(R)
KPN3	0(R)	7(R)	9(R)	0(R)
KPN4	0(R)	4(R)	11(R)	0(R)
KPN5	0(R)	10(R)	8(R)	0(R)
KPN6	0(R)	8(R)	11(R)	0(R)
KPN7	0(R)	11(R)	12(R)	0(R)
PA1	0(R)	0(R)	20(S)	0(R)
PA2	0(R)	0(R)	24(S)	0(R)
PA3	0(R)	0(R)	22(S)	0(R)
PA4	0(R)	0(R)	20(S)	0(R)
PA5	0(R)	0(R)	23(S)	0(R)
PA6	0(R)	0(R)	21(S)	0(R)

Note: KPN1–KPN7 are *K. pneumoniae*; PA1–PA6 are *P. aeruginosa*

Table 7: Summary of antimicrobial susceptibility patterns by organism

Bacterial Isolate	AMP (10 mcg)	TE (30 mcg)	GEN (10 mcg)	MET (5 mcg)
<i>E. coli</i>	100% (R)	100% (I)	100% (S)	100% (R)
<i>S. aureus</i>	100% (R)	100% (S)	100% (R)	100% (R)
<i>K. pneumoniae</i>	100% (R)	100% (R)	100% (R)	100% (R)
<i>P. aeruginosa</i>	100% (R)	100% (R)	100% (S)	100% (R)

AMP = Ampicillin; TE = Tetracycline; GEN = Gentamicin; MET = Methicillin; R = Resistant; I = Intermediate; S = Sensitive

Antibiotic Susceptibility Pattern

Tables 4–7 present zone of inhibition data and susceptibility interpretation for all isolates. Table 8 summarizes the overall susceptibility pattern per organism.

Resistance Findings by Organism

***E. coli*:** All 15 isolates showed complete resistance to AMP and MET. The 100% intermediate response to TE indicates reduced susceptibility and limited clinical utility. Gentamicin remained the most effective antibiotic, with 100% of isolates showing sensitivity.

***S. aureus*:** All 12 isolates were resistant to AMP, GEN, and MET. Methicillin resistance in 100% of isolates confirms the presence of MRSA. Tetracycline was the only effective antibiotic (100% sensitive), with zones ranging from 19 to 26 mm.

***K. pneumoniae*:** All seven isolates exhibited complete resistance (100%) to all four tested antibiotics — AMP, TE, GEN, and MET — indicating full MDR status. This is consistent with beta-lactamase production, efflux pump overexpression, and aminoglycoside-modifying enzyme activity.

***P. aeruginosa*:** All six isolates were resistant to AMP, TE, and MET. Although Gentamicin showed 100% sensitivity (zones 20–24 mm), the resistance to the remaining three antibiotics confirms MDR strains. Resistance to AMP and MET is consistent with the intrinsic resistance of Gram-negative organisms to these agents; Gentamicin sensitivity may reflect antibiotic class-specific mechanisms.

DISCUSSION

This investigation provides clear microbiological evidence that improperly managed biomedical waste from a tertiary-care hospital environment is a rich source of clinically significant and drug-resistant bacteria. The four organisms recovered — *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* — are among the most frequently reported pathogens in healthcare-associated infections globally (Bansod & Deshmukh, 2023; Medubi et al., 2006).

The predominance of *E. coli* (37.5%) aligns with earlier reports by Anitha and Jayraaj (2012) and Indira et al. (2012), who also identified this organism as the most frequently isolated species from hospital waste. Its recovery from diverse waste categories — urine bags, IV set tubes, used gloves, and spirit swabs — points to widespread faecal contamination and cross-contamination from clinical streams. Similarly, the 30% prevalence of *S. aureus* across waste types including syringes, blood-soaked cotton, and dressing materials echoes findings from Anitha et al. (2012) and Bansod and Deshmukh (2023), who attributed its environmental persistence to its robust survival characteristics.

The antimicrobial susceptibility data generated by this study are of particular public health concern. Universal

resistance to Ampicillin across all four organisms reflects the well-established loss of clinical utility for this drug, driven by beta-lactamase production and altered penicillin-binding proteins. This pattern has been consistently documented in hospital environments (C.D. Vieira et al., 2011; Shahzad et al., 2021).

The identification of MRSA characteristics in all 12 *S. aureus* isolates is especially alarming. MRSA strains are notoriously difficult to treat and contribute significantly to nosocomial morbidity and mortality. Their presence in discarded waste materials that are accessible to informal waste handlers and the general public underscores the environmental spillover risk of untreated BMW. Medubi et al. (2006) previously noted increasing MRSA prevalence on hospital waste dump sites and emphasized the need for routine surveillance.

Complete resistance to all tested antibiotics in *K. pneumoniae* confirms MDR status for this organism in the present setting. The resistance mechanisms likely include ESBL production (conferring AMP resistance), efflux pump upregulation (TE), and aminoglycoside-modifying enzymes (GEN). This finding is consistent with reports by Indira et al. (2012) and Awari et al. (2023), who documented increasing MDR among Gram-negative organisms in biomedical waste environments.

The retained sensitivity of *P. aeruginosa* to Gentamicin is noteworthy, though the resistance to the three remaining antibiotics confirms MDR categorization. This finding partially diverges from Shahzad et al. (2021), who documented high-level gentamicin resistance in hospital wastewater isolates, possibly reflecting differences in local antibiotic usage patterns and selective pressure at the study site.

Overall, the resistance patterns observed in the present study exceed those reported in earlier comparable studies, consistent with the global trajectory of escalating AMR driven by inappropriate antibiotic use, inadequate waste management, and environmental contamination. Biomedical waste thus functions not only as a source of direct infection but also as a vector for the environmental dissemination of resistance genes, creating a cycle that amplifies community-level AMR burden.

CONCLUSION

The results of this study confirm that biomedical waste from hospital environments harbours a wide spectrum of pathogenic bacteria, including MDR and MRSA strains. *E. coli* was the most frequently isolated organism (37.5%), while all four species — *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* — demonstrated high-level resistance to Ampicillin. MRSA was confirmed in all *S. aureus* isolates, and complete MDR was observed in *K. pneumoniae*. These findings have direct implications for infection control policy, waste management regulation, and antibiotic stewardship programmes.

Healthcare facilities must implement and audit rigorous BMW management protocols — including colour-coded segregation at source, on-site autoclaving or incineration, and training of all personnel involved in waste handling. Regular microbiological surveillance of waste streams should be incorporated into hospital infection control frameworks. Concurrently, rational antibiotic prescribing practices must be enforced to reduce the selection pressure that drives resistance emergence. Without these coordinated interventions, biomedical waste will continue to serve as an underappreciated driver of AMR dissemination into hospital and community environments alike.

Acknowledgement

We extend our sincere gratitude to the University Grants Commission for providing financial support as JRF to Mr. Yogesh for his research (UGC Ref. No. 231610040456). We also sincerely acknowledge the funding support in form of the Graduate Student research Award (GSRA) 2025 from the Botanical Society of America (BSA). We also extend our thanks to the Chairperson, Department of Botany, Kurukshetra University, Kurukshetra, and the Principal, I.B. (PG) College, Panipat, for their encouragement and institutional support.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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