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ANTIBACTERIAL ACTIVITY OF SWEET ORANGE (CITRUS SINENSIS) ON BACTERIA ISOLATED FROM WOUND INFECTIONS

Shruti Pandey[*1], Dr. Avinash Kumar Bhatia[2] and Dr. Raj Kumar[3]

[*1]PhD Scholar Microbiology, Desh Bhagat University, Mandi Gobindgarh (Punjab).
[2]Assistant Professor, Faculty of Agriculture and Life Sciences, Desh Bhagat University, Mandi Gobindgarh (Punjab).
[3]Professor, Department of Microbiology, Genesis Institute of Dental Science and Research, Firozpur (Punjab).

***Corresponding Author: Shruti Pandey**
PhD Scholar Microbiology, Desh Bhagat University, Mandi Gobindgarh (Punjab).

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ABSTRACT

Background: Wound infections remain a significant public health problem, contributing to delayed healing, prolonged hospitalization, and increased healthcare costs. The rising prevalence of antimicrobial resistance among wound pathogens has reduced the effectiveness of commonly used antibiotics, necessitating the exploration of alternative and adjunct antimicrobial agents. Medicinal plants and fruit-derived products have gained attention due to their bioactive compounds and potential antimicrobial properties. *Citrus sinensis* (sweet orange) is widely consumed and known to contain phytochemicals with antibacterial activity, making it a promising candidate for investigation against wound-infecting microorganisms. **Aim:** The present study aimed to determine the microbial profile of infected skin wounds and to evaluate the antibacterial activity of *Citrus sinensis* peel and juice extracts against bacterial pathogens isolated from wound infections. **Materials and Methods:** An observational laboratory-based study was conducted on 200 patients with clinically infected wounds attending the outpatient department of a tertiary care hospital in Punjab, India. Wound swabs were collected aseptically and processed for microbial culture and identification using standard microbiological and biochemical methods. Antibacterial activity of freshly prepared *C. sinensis* peel and juice extracts was assessed using the agar well diffusion method, and zones of inhibition were measured. Minimum inhibitory concentration (MIC) was determined by tube dilution for susceptible isolates. Antibiotic susceptibility testing of bacterial isolates was performed using standard disc diffusion techniques. Statistical analysis was carried out using SPSS, with significance set at $P < 0.05$. **Results:** Out of 200 wound samples, 103 (51.5%) were culture-positive. Monomicrobial infections predominated (74.76%), and Gram-negative bacteria were more common (67.44%) than Gram-positive bacteria (32.56%). *Staphylococcus aureus* (23.26%) was the most frequently isolated organism, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. *C. sinensis* juice extract exhibited stronger antibacterial activity than peel extract, with maximum inhibition observed against *S. aureus* (28.00 ± 2.10 mm) and *E. coli* (25.00 ± 4.20 mm). MIC results showed complete inhibition of *S. aureus* and *E. coli* at concentrations ≤10 mg/mL. Imipenem and amikacin were the most effective antibiotics, while high resistance was noted against ciprofloxacin. **Conclusion:** The study highlights the predominance of Gram-negative bacteria and increasing antibiotic resistance among wound pathogens. *Citrus sinensis* extracts, particularly juice, demonstrated significant antibacterial activity, suggesting their potential as complementary antimicrobial agents in the management of wound infections.

KEYWORDS: Wound infection; *Citrus sinensis*; Antibacterial activity; Antibiotic resistance; Medicinal plants.

INTRODUCTION

Wound infections remain a frequent reason for outpatient visits and hospital admissions because disruption of the skin barrier creates a direct gateway for microorganisms to invade underlying tissue, trigger inflammation, delay healing, and increase the risk of systemic complications. In routine care, even “simple” wounds can become clinically significant when microbial load increases, local perfusion is poor, or hygiene and dressing practices are inconsistent, leading to persistent discharge, pain, malodor, and impaired granulation. Surgical site infections (SSIs) deserve particular attention because

they represent a preventable cause of postoperative morbidity, prolonged hospital stay, additional antibiotic exposure, and higher treatment cost. The burden is especially relevant in resource-limited settings where delayed presentation, repeated procedures, and limited access to culture facilities can amplify complications. Recognizing these impacts, global guidance emphasizes evidence-based infection prevention and control, including wound hygiene, appropriate debridement, rational antibiotic use, and standard clinical decision pathways to reduce preventable infection and support safe recovery.[1]

From a microbiological standpoint, infected wounds are not sterile lesions; they are dynamic ecosystems shaped by the wound environment, host immune status, local oxygen tension, moisture, necrotic tissue, and prior antimicrobial exposure. Early colonizers may be replaced by opportunistic pathogens as the wound evolves, and clinically important infection occurs when microbial burden and virulence exceed host defenses, producing local signs (erythema, warmth, swelling, purulence) and sometimes systemic features (fever, leukocytosis). Both Gram-positive and Gram-negative organisms can dominate depending on wound type (traumatic, postoperative, diabetic foot, burn), anatomical site, chronicity, and healthcare contact. Importantly, polymicrobial interactions may intensify tissue damage and complicate therapy because different organisms contribute distinct enzymes, toxins, and resistance mechanisms. Therefore, culture-based identification and susceptibility testing remain critical for targeted therapy, while evaluation of alternative antibacterials is increasingly relevant where resistance or recurrent infection limits standard options.[2]

A major reason wound infections persist despite treatment is the ability of bacteria to form biofilms—organized microbial communities embedded within an extracellular matrix that adheres to tissue and protects organisms from host immunity and antimicrobials. Biofilms can reduce antibiotic penetration, slow bacterial metabolic activity (making drugs less effective), and promote tolerance and persistence, resulting in chronic inflammation and delayed epithelialization. Clinically, biofilm-associated wounds may show intermittent improvement followed by relapse, and the infection may appear "low-grade" yet stubborn, requiring repeated cleansing, debridement, and prolonged therapy. Biofilms also support mixed communities, allowing organisms to share nutrients and stress responses while maintaining a protective environment that is difficult to eradicate with conventional antibiotics alone. Understanding biofilms is therefore essential when exploring plant-derived antibacterials: if a natural extract can disrupt membrane integrity, interfere with quorum sensing, or weaken the extracellular matrix, it may complement standard therapy and improve wound outcomes.[3]

Among chronic wound categories, diabetic foot ulcers are particularly important because neuropathy, ischemia, hyperglycemia, and impaired immune function increase susceptibility to infection and limit healing. These infections often involve a broad range of pathogens, including staphylococci, Enterobacterales, and non-fermenters, and may progress from superficial colonization to deep tissue infection and osteomyelitis. Management therefore requires careful clinical assessment, culture-guided antibiotic therapy, off-loading, glycemic control, and timely surgical intervention when needed. Because unnecessary broad-spectrum antibiotics accelerate resistance and disrupt normal flora, guidelines emphasize obtaining appropriate specimens, selecting agents based on severity and likely pathogens, and narrowing treatment once laboratory results are available. In such settings, investigating affordable adjuncts—such as locally available Citrus sinensis preparations—becomes relevant, particularly if these products demonstrate reproducible in-vitro activity against common wound pathogens and can be standardized for safe use in future translational research.[4]

The therapeutic value of antibiotics is increasingly threatened by antimicrobial resistance (AMR), which is now recognized as a leading global health concern. Resistance increases the likelihood of treatment failure, prolonged infection, and complications, and it often forces clinicians to use more expensive or toxic second-line drugs. Wound infections contribute substantially to antibiotic consumption because empiric therapy is frequently initiated before culture results are available, especially when patients present late, have comorbidities, or show signs of spreading infection. This creates a cycle: frequent antibiotic exposure selects for resistant organisms, resistant organisms cause persistent infection, and persistent infection prompts further antibiotic use. Global analyses have quantified AMR burden across pathogens and syndromes, highlighting that resistant infections are associated with large numbers of deaths and disability, with especially high impact in low- and middle-income regions where access to diagnostics and optimized therapy can be limited. These realities support the scientific rationale for exploring complementary antibacterial sources—particularly plant-derived compounds—while still prioritizing stewardship and evidence-based antibiotic use.[5]

Because resistance trends vary across time and place, laboratory confirmation of susceptibility is central to rational therapy and meaningful research comparisons. The disk diffusion approach remains foundational for routine antimicrobial susceptibility testing because it is practical, comparatively low-cost, and suitable for many clinical laboratories. Its value comes from standardized inoculum preparation, controlled incubation, and interpretive criteria that allow zone diameters to be compared across isolates and studies. In thesis work that compares Citrus sinensis extracts with antibiotics,

applying standardized susceptibility principles is essential to avoid biased comparisons, ensure reproducibility, and support valid statistical analysis. Moreover, the same need for standardization extends to plant extracts: volume loaded into wells, concentration, solvent controls, and consistent incubation conditions must be maintained so that observed zones of inhibition reflect true antibacterial activity rather than technical variation.[6]

MATERIAL AND METHODS

An observational laboratory-based study was conducted in the Department of Microbiology, Desh Bhagat University (DBU), Punjab, with clinical samples obtained from the Outpatient Department (OPD) of Desh Bhagat Hospital, Amloh, Punjab. The work was planned to determine the microbial profile of infected skin wounds and to evaluate the antimicrobial activity of *Citrus sinensis* (sweet orange) extracts against the isolated bacterial pathogens. A total of 200 individuals presenting with clinical wound infection were included after obtaining informed consent. Participant details and sample identifiers were recorded using a structured format, and all procedures were performed following standard biosafety and aseptic guidelines to minimize contamination and ensure safe handling of clinical materials.

METHODOLOGY

Wound specimens were collected aseptically from infected skin wounds in the OPD using sterile swabs and/or appropriate sampling tools. Immediately after collection, samples were placed in sterile containers, labeled, and transported to the Microbiology Laboratory at DBU under aseptic conditions. On arrival, specimens were processed as early as possible; when immediate processing was not feasible, samples were stored at 4°C to preserve microbial viability until culture and further analysis.

Isolation of microorganisms from wound samples

Each clinical specimen was inoculated onto Mueller–Hinton agar (MHA) for bacterial isolation and onto potato dextrose agar (PDA) for fungal isolation. Spread plating was performed to obtain discrete colonies, and plates were incubated at 37°C for 48 hours. After incubation, morphologically distinct colonies were selected and sub-cultured to achieve purity. Pure isolates were maintained on agar slants and preserved at 4°C for subsequent identification and antimicrobial testing.

Identification and biochemical characterization of isolates

Bacterial isolates were identified using colony morphology, Gram staining, and a panel of standard biochemical tests. The tests performed included catalase, methyl red (MR), Voges–Proskauer (VP), triple sugar iron agar (TSIA), citrate utilization, sugar fermentation, and indole tests. Results from staining and biochemical profiles were interpreted collectively to characterize isolates at an appropriate taxonomic level using established microbiological criteria, and each isolate was assigned a laboratory code for traceability during downstream assays.

Collection and preparation of *Citrus sinensis* material

Fresh *Citrus sinensis* fruits were procured from the local market in Punjab and transported to the laboratory. The fruits were washed thoroughly with sterile distilled water and surface sterilized using 70% alcohol to reduce surface contaminants. Under aseptic conditions, peels were separated using a sterile knife, and juice was expressed into a sterile container. The pulp/juice fraction was filtered through a 0.45 µm Millipore filter to remove residual tissues and particulate matter. The resulting crude extract was prepared fresh and was used immediately without refrigeration as per the study protocol.

Screening of antimicrobial activity by agar well diffusion

The antimicrobial activity of *C. sinensis* extracts was screened using the agar well diffusion method on nutrient agar. Freshly prepared bacterial inocula were spread uniformly over the agar surface to create a confluent lawn. Wells were punched aseptically using a sterile glass borer, and 20 µL of peel/juice extract was dispensed into each well. Plates were incubated overnight at 37°C, and zones of inhibition were measured after 16–24 hours using a millimeter scale. Sterile distilled water or sterile ethanol was used as the negative control, and inhibition-zone diameters were recorded systematically for each isolate–extract combination.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined for extracts that produced a zone of inhibition ≥10 mm in the diffusion assay. Tube dilution was performed using nutrient broth as the medium, starting from the lowest tested active crude-extract concentration (20 mg/mL) and preparing serial dilutions to obtain 20 mg/mL and 10 mg/mL. For each concentration, 2 mL of extract was mixed with 2 mL of nutrient broth, and 100 µL of an overnight bacterial suspension standardized to 0.5 McFarland was inoculated. Positive and negative control tubes were included for comparison of turbidity. Tubes were incubated at 37°C for 24 hours and then examined visually for turbidity; absence of turbidity was considered indicative of inhibition. To confirm inhibition, non-turbid tubes were sub-cultured on MHA plates and incubated at 35±2°C for 18–24 hours. The lowest concentration that showed no visible growth and no growth upon subculture was recorded as the MIC.

Comparative testing with standard antibiotics

To benchmark the activity of fruit extracts, commercial antibiotic discs were used alongside extract testing on nutrient agar using standard diffusion principles. The

antibiotics included cotrimoxazole (25 µg), cefotaxime (30 µg), imipenem (10 µg), amoxycillin/clavulanate (20/10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), and other locally available standard antibiotics as required. Zone diameters were measured in millimeters and recorded to enable comparison between plant extracts and reference antimicrobial agents.

STATISTICAL ANALYSIS

All experimental observations were compiled and analyzed using SPSS (version 25.0). Zones of inhibition were expressed as mean values where appropriate, and differences among groups were assessed using one-way ANOVA followed by Tukey's post hoc multiple comparison test. Statistical significance was set at $P < 0.05$. MIC findings were summarized using descriptive statistics to present the distribution of inhibitory concentrations across isolates and extract types.

RESULTS

Table 1 (Demographic profile)

The demographic characteristics of the 200 wound-infected patients showed that the study population was predominantly middle-aged and elderly, with a mean age of 44.30 ± 15.20 years. The largest proportion of patients belonged to the 41–50 year age group (23%), followed by those aged 51–60 years (20%) and above 60 years (19%), indicating that wound infections were more common in older adults. Males constituted a higher proportion of cases (62%) compared to females (38%), suggesting a male predominance among wound-infected patients. A majority of patients were from rural areas (59%), highlighting a higher burden of wound infections in rural populations compared to urban residents (41%). With respect to education, most patients had secondary education (31%) or primary education (27%), while 16% were illiterate and 26% were graduates or above. Occupationally, homemakers (25%), farmers (24%), and laborers (23%) formed the major groups, reflecting that individuals involved in manual work or household activities were more frequently affected.

Table 2 (Clinical profile)

Analysis of clinical characteristics revealed that traumatic wounds were the most common type (36%), followed by post-operative wounds (23%). Diabetic foot ulcers accounted for 14% of cases, underscoring the role of diabetes as an important risk factor, while abscesses (13%), burn wounds (9%), and pressure sores (5%) were less frequent. The mean duration of wounds was 15.80 ± 10.40 days, with most patients presenting within 8–14 days (32%) or within the first week (29%). Lower limbs were the most commonly affected site (42%), followed by upper limbs (22%), indicating higher exposure of extremities to injury and infection. Regarding comorbidities, 41% of patients had no associated illness, whereas diabetes alone (28%) and hypertension (22%) were common, and 9% had both conditions. Prior antibiotic use was reported in 59% of patients, suggesting widespread empirical or previous treatment before presentation.

Table 3 (Culture outcome and growth pattern)

Out of the 200 wound samples processed, 103 (51.5%) were culture-positive, while 97 (48.5%) showed no microbial growth. Among the culture-positive samples, bacterial growth alone was observed in 90 cases (45%), fungal growth alone in 10 cases (5%), and mixed bacterial and fungal growth in 3 cases (1.5%). Nearly half of the samples (48.5%) did not yield growth on culture.

Table 4 (Infection pattern and bacterial isolate profile)

Among the 103 culture-positive samples, monomicrobial infections were more common (74.76%) than polymicrobial infections (25.24%), indicating that single-organism infections predominated. A total of 129 bacterial isolates were recovered, of which Gram-negative bacteria constituted the majority (67.44%), while Gram-positive bacteria accounted for 32.56%. *Staphylococcus aureus* was the most frequently isolated organism (23.26%), followed by *Klebsiella pneumoniae* (20.16%), *Pseudomonas aeruginosa* (18.60%), and *Escherichia coli* (17.83%). Coagulase-negative staphylococci (9.30%) and *Proteus* species (10.85%) were less common.

Table 5 (Antibacterial activity of Citrus sinensis extracts)

The antibacterial activity of *Citrus sinensis* peel and juice extracts, measured as zones of inhibition (ZOI), demonstrated variable efficacy against different bacterial species. For *Staphylococcus aureus*, the peel extract showed a mean ZOI of 20.00 ± 0.60 mm, while the juice extract exhibited stronger activity with a mean ZOI of 28.00 ± 2.10 mm. Similarly, *E. coli* was inhibited by both extracts, with the peel extract showing a mean ZOI of 22.20 ± 3.10 mm and the juice extract showing 25.00 ± 4.20 mm. *Klebsiella pneumoniae* showed limited susceptibility to the peel extract (mean ZOI 11.60 ± 1.20 mm) and no measurable response to the juice extract. *Pseudomonas aeruginosa* exhibited moderate inhibition by both extracts, with greater activity observed for the juice extract (22.00 ± 2.50 mm) compared to the peel extract (18.00 ± 0.00 mm).

Table 6 (MIC distribution)

Minimum inhibitory concentration (MIC) analysis revealed that both peel and juice extracts were effective at low concentrations against *S. aureus* and *E. coli*. For the peel extract, all *S. aureus* isolates (100%) were inhibited at concentrations ≤10 mg/mL, with an average MIC of 0.63 mg/mL, while *E. coli* isolates showed complete inhibition at the same concentration range with an average MIC of 1.25 mg/mL. Similar results were observed for the juice extract, where all *S. aureus* isolates were inhibited at ≤10 mg/mL (average MIC

0.63% v/v) and all *E. coli* isolates at ≤10 mg/mL (average MIC 1.25% v/v).

Table 7 (Antibiotic susceptibility patterns)
The antibiotic susceptibility profiles revealed varying degrees of resistance among the bacterial isolates. *Staphylococcus aureus* showed highest sensitivity to imipenem (80%) and amikacin (73.33%), while high resistance was observed against cefotaxime (73.33%) and ciprofloxacin (60%). *Escherichia coli* exhibited good sensitivity to imipenem (86.96%) and amikacin (78.26%), but showed high resistance to ciprofloxacin (69.57%). Similarly, *Klebsiella pneumoniae* isolates were most sensitive to imipenem (80.77%) and amikacin (73.08%), while resistance to ciprofloxacin was common (65.38%). *Pseudomonas aeruginosa* also demonstrated highest susceptibility to imipenem (79.17%) and amikacin (70.83%), with notable resistance to ciprofloxacin (66.67%).

Table 1: Demographic profile of wound-infected patients (N = 200).

Variable	Category	n	%
Age (years)	Mean ± SD	44.30 ± 15.20	
	≤20	12	6.00
	21–30	28	14.00
	31–40	36	18.00
	41–50	46	23.00
	51–60	40	20.00
	>60	38	19.00
Gender	Male	124	62.00
	Female	76	38.00
Residence	Rural	118	59.00
	Urban	82	41.00
Education status	Illiterate	32	16.00
	Primary	54	27.00
	Secondary	62	31.00
	Graduate and above	52	26.00
Occupation	Farmer	48	24.00
	Laborer	46	23.00
	Homemaker	50	25.00
	Employed	34	17.00
	Student	22	11.00

Table 2: Clinical profile of wound-infected patients (N = 200).

Variable	Category	n	%
Type of wound	Traumatic	72	36.00
	Post-operative	46	23.00
	Diabetic foot ulcer	28	14.00
	Abscess	26	13.00
	Burn wound	18	9.00
	Pressure sore	10	5.00
Duration (days)	Mean ± SD	15.80 ± 10.40	—
	≤7	58	29.00
	8–14	64	32.00
	15–30	50	25.00
	>30	28	14.00
Wound site	Lower limb	84	42.00
	Upper limb	44	22.00
	Trunk	28	14.00
	Head/neck	14	7.00
	Perineum/groin	30	15.00
Comorbidity	None	82	41.00
	Diabetes	56	28.00
	Hypertension	44	22.00
	Diabetes + HTN	18	9.00
Prior antibiotic use	Yes	118	59.00
	No	82	41.00

Table 3: Culture outcome and growth pattern on media (N = 200).

Parameter / Growth pattern	n	%	Average
Total samples	200	100.00	—
Culture-positive	103	51.50	51.57
Culture-negative	97	48.50	48.43
Bacterial growth only	90	45.00	45.00
Fungal growth only	10	5.00	5.00
Mixed (bacterial + fungal)	3	1.50	1.50
No growth	97	48.50	48.50

Note: Culture-positive samples include bacterial, fungal, and mixed growth. Subsequent analysis focused on bacterial isolates only.

Table 4: Infection pattern and bacterial isolate profile (n = 103 culture-positive; total isolates = 129).

Section	Category / Organism	n	%	Average
Infection pattern among culture-positive (n = 103)	Monomicrobial	77	74.76	72.90
	Polymicrobial	26	25.24	27.10
Gram reaction (total isolates = 129)	Gram-positive	42	32.56	33.00
	Gram-negative	87	67.44	67.00
Distribution of bacterial isolates (total = 129)	*Staphylococcus aureus*	30	23.26	21.94
	CoNS	12	9.30	8.60
	Escherichia coli	23	17.83	16.79
	Klebsiella pneumoniae	26	20.16	18.63
	Pseudomonas aeruginosa	24	18.60	16.93
	Proteus spp.	14	10.85	10.00
Final biochemical identification of isolates	*S. aureus* (Tested/Identified)	30/30	100.00	100.00
	CoNS (Tested/Identified)	12/12	100.00	100.00
	E. coli (Tested/Identified)	23/23	100.00	100.00
	K. pneumoniae (Tested/Identified)	26/26	100.00	100.00
	P. aeruginosa (Tested/Identified)	24/24	100.00	100.00
	Proteus spp. (Tested/Identified)	14/14	100.00	100.00

Table 5: Antibacterial activity of *Citrus sinensis* extracts (ZOI, mm).

Organism	Peel extract (Min)	Peel extract (Max)	Peel extract (Mean ± SD)	Juice extract (Min)	Juice extract (Max)	Juice extract (Mean ± SD)	Peel ZOI	Juice ZOI	Mean difference	p-value
S. aureus	19.40	20.60	20.00 ± 0.60	25.90	30.10	28.00 ± 2.10	20.00	28.00	8.00	NR
E. coli	19.10	25.30	22.20 ± 3.10	20.80	29.20	25.00 ± 4.20	22.20	25.00	2.80	NR
K. pneumoniae	10.40	12.80	11.60 ± 1.20	—	—	NR	—	—	—	—
P. aeruginosa	18.00	18.00	18.00 ± 0.00	19.50	24.50	22.00 ± 2.50	18.00	22.00	4.00	NR

Table 6: MIC distribution of *Citrus sinensis* peel and juice extracts.

Extract	Organism	≤10 mg/mL n (%)	20 mg/mL n (%)	Average MIC
Peel extract	*S. aureus*	30 (100.00)	0 (0.00)	0.63 mg/mL
Peel extract	*E. coli*	23 (100.00)	0 (0.00)	1.25 mg/mL
Juice extract	*S. aureus*	30 (100.00)	0 (0.00)	0.63 % v/v
Juice extract	*E. coli*	23 (100.00)	0 (0.00)	1.25 % v/v

Table 7: Antibiotic susceptibility patterns of bacterial isolates.

Organism (n)	Antibiotic	Sensitive n (%)	Resistant n (%)	p-value
Staphylococcus aureus (30)	Ciprofloxacin	12 (40.00)	18 (60.00)	0.041
	Amikacin	22 (73.33)	8 (26.67)	0.003
	Gentamicin	16 (53.33)	14 (46.67)	0.218
	Imipenem	24 (80.00)	6 (20.00)	<0.001

	Cefotaxime	8 (26.67)	22 (73.33)	0.007
Escherichia coli (23)	Ciprofloxacin	7 (30.43)	16 (69.57)	0.034
	Amikacin	18 (78.26)	5 (21.74)	0.001
	Gentamicin	11 (47.83)	12 (52.17)	0.612
	Imipenem	20 (86.96)	3 (13.04)	<0.001
Klebsiella pneumoniae (26)	Ciprofloxacin	9 (34.62)	17 (65.38)	0.029
	Amikacin	19 (73.08)	7 (26.92)	0.004
	Gentamicin	13 (50.00)	13 (50.00)	1.000
	Imipenem	21 (80.77)	5 (19.23)	<0.001
Pseudomonas aeruginosa (24)	Ciprofloxacin	8 (33.33)	16 (66.67)	0.038
	Amikacin	17 (70.83)	7 (29.17)	0.006
	Gentamicin	12 (50.00)	12 (50.00)	1.000
	Imipenem	19 (79.17)	5 (20.83)	<0.001

DISCUSSION

In the present study (N = 200), wound infection was concentrated in middle-to-older age groups (mean age 44.30 ± 15.20 years), with the highest frequency in 41–50 years (23%), and a clear male predominance (62%). This demographic pattern is consistent with large surgical-wound cohorts where adult and older patients form the major at-risk population, likely due to higher exposure to trauma, comorbidities, and healthcare contact. For example, Giacometti et al. (2000) evaluated 676 patients with suspected surgical wound infections and documented substantial culture-confirmed infection burden in a similarly adult-dominant population, supporting that wound infections are predominantly an adult clinical problem across settings.[7]

Clinically, traumatic wounds were the leading presentation in this study (36%), followed by post-operative wounds (23%), with a mean wound duration of 15.80 ± 10.40 days and most patients presenting within 8–14 days (32%). This distribution aligns with the concept that acute wounds and post-procedure wounds drive a major proportion of infected wound visits. In post-operative–specific settings, however, the microbiology burden can be concentrated within narrower clinical groups. In a post-caesarean wound infection analysis, Das et al. (2024) reported an SSI incidence of 8.72% (24 infections among 275 caesarean cases) and observed that organism patterns and antibiograms are strongly shaped by the procedure and peripartum context—highlighting why the broader case-mix in the present OPD-based study yields a different clinical profile dominated by traumatic and mixed wound categories.[8]

Microbiologically, the present study showed 51.5% culture positivity (103/200), while 48.5% yielded no growth. A comparable but somewhat lower growth proportion has been reported in high-throughput tertiary-care wound surveillance: Mansoor et al. (2024) found bacterial growth in 43.9% of 4,378 wound specimens (1,921 positives), emphasizing that clinically "infected" wounds may not always be culture-positive due to prior antibiotics, sampling depth, or non-bacterial etiologies.[9]

Among culture-positive cases in this study, monomicrobial infections (74.76%) were markedly more common than polymicrobial infections (25.24%), and overall isolates were predominantly Gram-negative (67.44%) rather than Gram-positive (32.56%). This Gram-negative predominance is consistent with multiple wound-surveillance reports. For instance, Mansoor et al. (2024) reported 73.5% Gram-negative bacilli among isolates, reinforcing that Gram-negative pathogens frequently dominate wound bacteriology in many hospital and regional contexts.[10]

The organism distribution in the present study—S. aureus (23.26%), K. pneumoniae (20.16%), P. aeruginosa (18.60%), E. coli (17.83%), CoNS (9.30%), and Proteus spp. (10.85%)—shows a mixed flora where both classic skin pathogens and enteric/non-fermenters contribute substantially. In contrast, procedure-specific cohorts can show heavier skew toward a single organism: Das et al. (2024) reported S. aureus 45.45% (10/22 isolates), with E. coli 18.18% and Klebsiella 18.18% in post-caesarean infections—higher S. aureus dominance than in the present mixed-wound OPD population, where Gram-negative organisms collectively predominate.[11]

Antibiotic susceptibility in this study demonstrated that carbapenem and aminoglycoside activity remained comparatively strong across key pathogens (e.g., imipenem sensitivity: S. aureus 80%, E. coli 86.96%, K. pneumoniae 80.77%, P. aeruginosa 79.17%; amikacin sensitivity: 70.83–78.26% across major Gram-negatives), while fluoroquinolone performance was weaker (ciprofloxacin resistance 60–69.57% across several organisms). When compared with institutional Gram-negative antibiograms, these sensitivities can vary substantially by setting and species mix. Kakhandki et al. (2020), analyzing 736 Gram-negative isolates from SSTIs, reported much lower overall sensitivity rates for key agents—imipenem 51%, amikacin 43%, and ciprofloxacin 34%—underscoring that local resistance ecology can markedly reduce empiric options relative to the higher carbapenem/amikacin activity observed in the present study.[12]

Regarding the plant extract findings, Citrus sinensis showed meaningful antibacterial activity in this study, with generally stronger inhibition by juice than peel for most tested organisms: for S. aureus, peel 20.00 ± 0.60 mm vs juice 28.00 ± 2.10 mm; for E. coli, peel 22.20 ± 3.10 mm vs juice 25.00 ± 4.20 mm; and for P. aeruginosa, peel 18.00 ± 0.00 mm vs juice 22.00 ± 2.50 mm. Prior experimental work supports orange peel antibacterial effects but often with smaller zones depending on extraction and dosing. Oikeh et al. (2020) reported, at 200 µg/mL peel extract, zones of 14 mm (S. aureus) and 13 mm (E. coli) (and 14 mm for P. aeruginosa)—lower than the present study's mean peel ZOI for S. aureus and E. coli, which may reflect differences in extract preparation (fresh crude fractions here vs ethanol extracts there), inoculum, and assay conditions.[13]

MIC findings in the present study further indicate strong inhibition at low tested concentrations for the two evaluated organisms: 100% of S. aureus and E. coli isolates were inhibited at ≤10 mg/mL for peel (average MIC 0.63 mg/mL for S. aureus; 1.25 mg/mL for E. coli), with parallel complete inhibition at ≤10 (reported as % v/v) for juice. Comparable peel-extract MIC ranges have been documented in controlled experiments: Oikeh et al. (2020) reported MIC values spanning 12.5–100 µg/mL for peel extracts against multiple organisms, including 12.5 µg/mL against S. aureus for fresh peel extract—showing that, under purified solvent-extract conditions, MICs can appear lower than crude clinical-testing preparations, again emphasizing the influence of extraction solvent, standardization, and concentration expression on MIC comparability.[14]

Finally, integrating resistance and phytotherapy relevance, the present antibiogram highlights clinically important resistance signals—e.g., cefota xime resistance 73.33% in S. aureus and ciprofloxacin resistance 65–70% in major Gram-negative organisms—supporting the need for continued local surveillance and stewardship. Large epidemiologic surgical-wound datasets similarly demonstrate that wound infections are frequently culture-confirmed and often polymicrobial, with a wide pathogen spectrum dominated by aerobes; Giacometti et al. (2000) reported bacteria isolated from 614/676 patients, with multiple agents in 343 cases and leading pathogens including S. aureus 28.2% and P. aeruginosa 25.2%—figures that contextualize the present study's mixed pathogen ecology (S. aureus 23.26%, P. aeruginosa 18.60%) and reinforce why both effective antibiotics (guided by local sensitivity) and exploration of adjunct antimicrobial sources (e.g., Citrus extracts) remain relevant in wound infection management.[15]

CONCLUSION

This study demonstrates that wound infections were more common among middle-aged and elderly patients and were predominantly caused by Gram-negative bacteria, with *Staphylococcus aureus* remaining the most frequent single isolate. High resistance to commonly used antibiotics, particularly ciprofloxacin, highlights the growing challenge of antimicrobial resistance in wound management. *Citrus sinensis* peel and juice extracts showed significant antibacterial activity against major wound pathogens, with juice extract exhibiting superior efficacy. These findings suggest that *C. sinensis* may serve as a promising adjunct or alternative source of antimicrobial agents, warranting further investigation and standardization for clinical application.

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