Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

ANTIBIOTIC SUSCEPTIBILITY PROFILING OF BACTERIA ISOLATED FROM SURFACE OF VALIDATOR MACHINES INSTALLED IN BUS RAPID TRANSIT (BRT), PESHAWAR

Sareer Ahmad

Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar. sareermicrobes@gmail.com

Rimsha Shahroom

Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar. rimshashahroom6@gmail.com

Farid Ahmad

faridahmad3269828@gmail.com

Khudija Ghani*

Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar. Corresponding Author Email: khudija.siahs@suit.edu.pk

Dr. Shabir Ahmad

Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar. Shabir.biotech@suit.edu.pk

ABSTRACT

Validator machines for tickets validation are fundamental parts of modern public transportation systems, serving as key junctions for fare collection and passenger validation. Despite their significance in enhancing the functionality of public transportation systems, validator machines are susceptible to surface contamination and microbial colonization, posing potential health risks to passengers and transit personnel. There, this study aimed to assess the prevalence and antibiotic susceptibility profiles of bacteria isolated from the surfaces of validator machines installed in Bus Rapid Transit (BRT) stations in Peshawar. A total of 124 swab samples were collected from the in and out validator machines (4 validator machines in each station) across 31 BRT stations in Peshawar. The swab samples were cultured on growth media and the isolated bacterial colonies were identified through biochemical tests and gram staining. Out of 124 samples, 65 (52.41 %) of the bacterial isolates were gram positive and 59 (47.58 %) of the isolates were gram

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

negative. Several gram positive and gram negative bacterial isolates including *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Klebsiella* and *Proteus* species were identified. The antibiotic susceptibility testing was performed on a total of 65 gram positive isolates including 57 *Staphylococcus aureus* and 8 *Enterococcus* species. Among them, 22 (33.84 %) of the *S. aureus* isolates were identified as methicillin resistant *S. aureus* (MRSA). The antibiotic susceptibility testing was performed for a total of 59 gram negative bacterial isolates. All the gram negative isolates were MDR, showing resistance to multiple drugs. Piperacillin/Tazobactam was the most sensitive antibiotic against the tested species.

Keywords: Validator machines, Bus Rapid Transit (BRT), Gram positive, Gram negative.

INTRODUCTION

Validator machines for tickets validation are fundamental parts of modern public transportation systems, serving as key junctions for fare collection and passenger validation (Ayare *et al.*, 2023). The implementation of efficient transit systems is essential for mitigating transportation challenges and improving overall mobility in densely populated urban areas, where traffic congestion and overcrowding are common (Busari *et al.*, 2021). The introduction of transportation networks has revolutionized public transit, offering commuters a reliable and easy mode of travel. Validator machines strategically installed at transportation stations ensure the smooth functioning of these transit systems, allowing passengers to easily access and utilize public transportation services (Lu *et al.*, 2020).

These validator machines play a crucial role in simplifying the boarding process and ensuring fare compliance, thereby contributing to the efficiency and sustainability of urban transit networks (Hasan *et al.*, 2023). By enabling passengers to validate their tickets or smart cards before boarding trains or buses, these devices help in reducing fare evasion and minimizing revenue loss, while also promoting a more organized and hassle-free commuting experience. Moreover, validator machines facilitate the integration of various fare payment systems, including contactless smart cards and mobile ticketing applications, further enhancing convenience and accessibility for commuters (Ayare *et al.*, 2023; Golub *et al.*, 2021).

Despite their significance in enhancing the functionality of public transportation systems, validator machines are susceptible to surface contamination and microbial colonization, posing potential health risks to passengers and transit personnel (Chebon & Sonoiya. 2019). The constant handling of these devices by a diverse range of commuters, coupled with the high volume of traffic passing through transit stations, creates an environment conducive to the accumulation and transmission of bacteria and other microorganisms (Wen *et al.*, 2020; Budzynski *et al.*, 2021).

Research on contamination in transit environments has highlighted the presence of microorganisms on commonly touched surfaces, amplifying concerns about public health and hygiene (Bhardwaj *et al.*, 2023; Njoku & Joseph. 2022) . Studies conducted globally have consistently identified bacteria, viruses and other pathogens on surfaces such as handrails, seats, and ticketing machines within transit stations (Danko *et al.*, 2021). These findings underscore the potential for surface contamination to serve as a reservoir for infectious agents, posing risks of transmission to commuters and transit staff (Cave *et al.*, 2021). Validator machines,

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

in particular, stand out as prominent sites for microbial colonization due to their frequent interaction with passengers (Kimenko *et al.*, 2021). The continuous exposure to human touch, along with the environmental factors such as humidity and temperature fluctuations, creates favorable conditions for the proliferation of microorganisms on these surfaces (Budzynski *et al.*, 2021).

Despite efforts to uphold cleanliness standards through routine cleaning and disinfection protocols, transit stations face inherent challenges in maintaining optimal hygiene levels (Zhang *et al.*, 2023; Tirachini & Cats. 2020) . The dynamic nature of transit environments, characterized by the constant influx and egress of passengers, complicates sanitation efforts (Zahraei & Houshmandfarzaneh. 2023). Moreover, a large volume of foot traffic passing through stations further exacerbates the difficulty of controlling surface contamination (Ainsalu *et al.*, 2018). Despite the implementation of cleaning schedules, validator machines may still harbor bacteria and other pathogens due to the rapid turnover of passengers, making it challenging to effectively mitigate the risk of transmission (Budzynski *et al.*, 2021; Arora *et al.*, 2021).

Ensuring the cleanliness of validator machines and other high-touch surfaces in transit stations requires a multifaceted approach that addresses both infrastructure and behavioral factors (Force. 2020). Enhanced cleaning protocols, including more frequent disinfection of surfaces and the use of antimicrobial coatings, can help reduce microbial colonization on validator machines (Kalb *et al.*, 2022). Additionally, public awareness campaigns emphasizing the importance of hand hygiene and responsible behavior can empower commuters to contribute to a cleaner transit environment (Lazini *et al.*, 2024; Javan *et al.*, 2023). By adopting a step towards maintaining hygiene standards, transit authorities can mitigate the risk of surface contamination and promote safer travel experiences for passengers and staff alike (Taylor & Ding. 2021; Ak & Demir. 2023).

Moreover, the implementation of innovative technologies, such as ultraviolet (UV) disinfection systems or electrostatic sprayers, can complement traditional cleaning methods by providing more thorough and efficient disinfection of validator machines and other high-touch surfaces (Obregon & Rodrigoez. 2021; Sidorchuk *et al.*, 2020). These advanced techniques offer the advantage of targeting hard-to-reach areas and achieving higher levels of microbial reduction, thereby enhancing the overall effectiveness of sanitation efforts (Bharti *et al.*, 2022; Arunwuttipong *et al.*, 2021) . Integrating such technologies into routine cleaning can significantly bolster the cleanliness and hygiene of transit stations, further minimizing the risk of surface contamination (Hui *et al.*, 2023).

In addition to infrastructure improvements, fostering a culture of cleanliness and hygiene among commuters is paramount in ensuring the long-term success of sanitation initiatives (Tennakoon *et al.*, 2020). Public awareness campaigns can play a pivotal role in educating passengers about the importance of hand hygiene practices, such as regular handwashing with soap and water or the use of hand sanitizers, especially after touching communal surfaces (Nicolaides *et al.*, 2020; Omari *et al.*, 2022). By promoting responsible behavior and emphasizing individual accountability for maintaining a clean environment, transit authorities can empower passengers to actively contribute to the prevention of surface contamination and the spread of

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

infectious diseases (Prats. 2020; White *et al.*, 2013).

Furthermore, collaboration between transit agencies, local health authorities and community organizations is essential for developing comprehensive hygiene management strategies personalized to the unique needs and challenges of transit environments (Moh. 2017). By leveraging collective expertise and resources, stakeholders can devise targeted interventions, implement best practices, and monitor outcomes to continuously improve hygiene standards across transit systems (Mughal. 2019; Bibri & Krogstie. 2020). This collaborative approach fosters a sense of shared responsibility and commitment to safeguarding public health, reinforcing the importance of maintaining cleanliness in transit stations as a collective effort (Meyer & Elrahman. 2019).

Ultimately, ensuring the cleanliness of validator machines and other high-touch surfaces in transit stations is not merely a matter of hygiene but also a fundamental aspect of public safety and well-being (Ampu *et al.*, 2019). By adopting a holistic approach that integrates infrastructure enhancements, public education initiatives, and collaborative partnerships, transit authorities can create a conducive environment for promoting hygiene and mitigating the risk of surface contamination (Apata *et al.*, 2023). Through sustained efforts and collective engagement, transit stations can become safer and more sanitary spaces, fostering confidence among passengers and staff alike and promoting a positive travel experience for all (Abaitua Knight. 2023).

METHODOLOGY

Study Design

The current study was a cross sectional study.

Study Area

This study was conducted in the Microbiology Laboratory of Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar.

Sampling Strategy

The sampling strategy involved the collection of swab samples from validator machines within Bus Rapid Transit (BRT) stations. For each station, a systematic sampling approach was used to select 2 in and 2 out validator machines.

Selection of Stations

Thirty one BRT stations across Peshawar were selected for sampling.

Sample Collection

Sterile swabs were used to collect samples from the surfaces of BRT validator machines. Before sampling, the surfaces of the validator machines were cleaned with alcohol wipes to remove any surface contaminants. Then, the surface of each validator machine was swabbed to ensure comprehensive sampling. Swabs were moistened with sterile normal saline. Collected samples were labeled with the location and time of collection and transported to the Microbiology Laboratory of Institute of Allied Health Sciences, Sarhad University of Science and Information Technology.

Sample Processing

In the laboratory, the swabs were immediately cultured on nutrient, macConkey, blood, mannitol salt and cysteine, lactose and electrolyte-deficient (CLED) agar, then

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

incubated at 37 °C for 24 hours. After 24 hours, the colony morphology *i-e.*, color, shape and general appearance was examined (Wali *et al.*, 2022).

Bacterial Identification

The isolated bacterial pathogens were identified on the basis of morphology, gram staining and several biochemical tests according to standard microbiological techniques.

Morphological Identification

The isolated colonies were then sub cultured on macConkey, blood and mannitol salt agar and incubated at 37 °C for 24 hours. After incubation, the plates were examined for morphological characteristics (Kabanangi *et al.*, 2021).

Gram Staining

The isolates were further identified through gram staining. For accurate results, 24 hours fresh cultured plates were used. A single and isolated bacterial colony was picked with the help of a sterilized loop for preparing smear on a sterile glass slide. The slide was first heat fixed. The slide was then stained with the crystal violet dye followed by treating with gram's iodine solution. Rapid decolorization was performed with the help of ethanol or acetone. It was then followed by counterstaining with safranin (Kabanangi *et al.*, 2021).

Biochemical Tests

Several biochemical tests including indole, citrate, triple sugar iron (TSI), oxidase and urease test was performed to identify the bacterial species isolated.

Indole Test

The isolated bacteria were first inoculated in peptone water and then incubated for 24 hours at 37°C. The xylene was added to the peptone water and mixed thoroughly to remove indole. After this, Kovac reagent was added to the tubes. The formation of a pink colored ring on the surface of the tube showed positive result (Mohammed *et al.*, 2023).

Citrate Test

The isolated bacterial colonies were gently inoculated on simmon citrate agar slants using a sterilized wire loop and incubated at 37°C for 24 hours. After incubation, the slants were observed for a color change from green to blue which showed positive results (Inya *et al.*, 2022).

Triple Sugar Iron (TSI) Test

TSI medium slants were prepared in a test tube. A single bacterial colony was picked via a sterilized wire loop and then streaked across the whole surface of the slant and then butt. The tubes were incubated for 24 hours at 37 °C. After 24 hours of incubation, the tubes were examined (Stanley, 2022).

Oxidase Test

Oxidase reagent was prepared by mixing oxidase in distilled water. Filter paper was soaked in it. A single isolated bacterial colony from 24 hours fresh culture was picked with the help of a loop and rubbed over the moist filter paper. The appearance of dark purple or blue color within 10 seconds showed positive result (Eze *et al.*, 2021).

Catalase Test

Few drops of hydrogen peroxide were added to the slide. Overnight fresh bacterial colony was placed on a slide using a sterile loop. The rapid formation of bubbles

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

caused by the breakdown of H_2O_2 showed positive result (Bhalla *et al.*, 2018).

DNase Test

DNase media was prepared and isolated bacterial colonies were cultured on the test area and incubated for 24 hours at 37°C. After 24 hours of incubation, the plates were rinsed with 1 N HCl. Clear zones surrounding the bacterial colonies indicate positive result (Arjyal & Neupane, 2020).

Antibiotic Susceptibility Testing

The confirmed bacterial isolates were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method against frequently used antibiotics belonging to various groups (CLSI, 2022). Inoculums for susceptibility testing was prepared by suspending three to five well isolated colonies from nutrient agar plate into an eppendorf tube containing normal saline. The turbidity of the inoculum was adjusted by comparing it with 0.5 McFarland standard. Bacterial lawn was prepared by dipping a sterile cotton swab into the eppendorf tube. Excess fluid was squeezed against the wall of the eppendorf tube. The swab was streaked onto the Muller Hinton agar plate and left for five minutes to dry. The antibiotics were then aseptically placed on MHA plate and incubated for 24 hours at 37°C. After incubation, the zones of inhibition were measured with the help of scale (Al-Ezzy *et al.*, 2021).

RESULTS

Total Samples Processed

A total of 124 swab samples were collected from the in and out validator machines (4 validator machines in each station) across 31 BRT stations in Peshawar.

Initial Bacterial Characterization

Out of 124 samples, 65 (52.41 %) of the bacterial isolates were gram positive and 59 (47.58
%) of the isolates were gram negative. The isolated bacteria with their name and number are shown in table 1.

Table. 1: ***Initial Bacterial Characteriation***

S. No.	Predicted Bacteria	No. of Isolates	Isolation Rate
1.	*Pseudomonas aeruginosa*	19	15.32 %
2.	*Staphylococcus aureus*	57	45.96 %
3.	*Proteus* spp.	17	13.70 %
4.	*Escherichia coli*	13	10.48 %
5.	*Enterococcus* spp.	8	6.45 %
6.	*Acinetobacter baumannii*	6	4.83 %
7.	*Klebsiella pneumoniae*	4	3.22 %
Total		124	100 %

Identification of the isolated bacteria

Morphological Identification

The collected samples when cultured on nutrient, macConkey, blood, mannitol salt and CLED agar formed various colonies. *Pseudomonas* formed smooth colonies with flat margins. *Staphylococcus aureus* formed bright yellow colored colonies on MSA media. *Klebsiella* formed pink color mucoid colonies on macConkey agar media. *Proteus* species when cultured on nutrient agar plate formed glistening small colonies

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

in swarming position and smooth, dull colonies on macConkey agar, while it formed translucent blue color colonies on CLED agar. *Acinetobacter* formed smooth, round and opaque colonies with clear margins on blood agar media. *Enterococcus* formed small and dark grey color colonies on MSA media. while, *E. coli* formed pink, flat, mucoid colonies on macConkey agar media.

Gram Staining

Several gram positive and gram negative bacterial isolates including *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Klebsiella* and *Proteus* species were identified. The isolated bacteria with their colony morphology are depicted in table 2.

Table 2: ***Several gram positive and gram negative bacterial isolates with their colony morphology***

S. No.	Sample	Gram Stain Reaction	Morphological characteristics	Predicted bacteria
1.	Swab	+ ve	Purple color cocci or spherical shape	*Staphylococcus* spp.
2.	Swab	- ve	Pink color rods	*Pseudomonas* spp.
3.	Swab	- ve	Pink color rods/coccobacillus	*Acinetobacter* spp.
4.	Swab	- ve	Pink color rods	*Klebsiella* spp.
5.	Swab	- ve	Pink color rods	*Proteus* spp.
6.	Swab	- ve	Pink color rods	*E. coli*
7.	Swab	+ ve	Purple color coccobacillus	*Enterococcus* spp.

Biochemical Tests

Several biochemical tests performed to identify the isolated gram-positive and gram-negative bacteria with their results are depicted in table 3. Different bacterial species were identified based on various biochemical tests.

Table 3: Several biochemical tests with their results

Biochemical Tests						
Predicted Bacteria	Indole	Citrate	TSI	Oxidase	Catalase	DNase
Staphylococcus spp.	--------	--------	--------	--------	--------	+ ve
Pseudomonas spp.	-ve	+ ve	--------	+ ve	+ ve	--------
Acinetobacter spp.	-ve	+ ve	+ ve	-ve	+ ve	--------
Klebsiella spp.	+ ve	+ ve	-ve	--------	--------	--------
Proteus spp.	-ve	+ ve	+ ve	-ve	+ ve	--------
MRSA	--------	--------	--------	--------	--------	+ ve
E. coli	+ ve	-ve	+ ve	-ve	-ve	--------
Enterococcus spp.	+ ve	-ve		-ve	-ve	--------

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Antibiotic Susceptibility Testing

Antibiotic Susceptibility of Gram-Positive Bacteria

The antibiotic susceptibility testing was performed on a total of 65 gram positive isolates including 57 *Staphylococcus aureus* and 8 *Enterococcus* species. Among them, 22 (33.84 %) of the *S. aureus* isolates were identified as methicillin resistant *S. aureus* (MRSA). The susceptibility results are listed in table 4.

Table 4. Antibiotic susceptibility testing of gram-positive bacterial isolates

S. No.	Antibiotics	Concentration	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
1	Vancomycin	30 µg	63 (96.92 %)	------	2 (3.07 %)
2	Ampicillin	30 µg	------	------	65 (100 %)
3	Imipenem	30 µg	------	2 (3.07 %)	63 (96.92 %)
4	Gentamicin	10 µg	23 (35.38 %)	-------	42 (64.61 %)
5	Linezolid	30 µg	61 (93.84 %)	1 (1.53 %)	3 (4.61 %)
6.	Ciprofloxacin	5 µg	4 (6.15 %)	17 (26.15 %)	44 (67.69 %)
7.	Oxacillin	30 µg	43 (66.15 %)	-------	22 (33.84 %)
8.	Cefoxitin	30 µg	43 (66.15 %)	-------	22 (33.84 %)
9.	Clindamycin	2 µg	57 (87.69 %)	-------	8 (12.30 %)

Antibiotic Susceptibility of Gram-Negative Bacteria

The antibiotic suscpetibility testing was performed for a total of 59 gram negative bacterial isolates. All the gram negative isolates were MDR, showing resistance to multiple drugs. Piperacillin/Tazobactam was the most sensitive antibiotic against the tested species. The results are listed in table 5.

Table 5. Antibiotic susceptibility testing of gram-negative bacterial isolates

S. No.	Antibiotics	Concentration	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
1.	Ciprofloxacin	5 µg	5 (8.47 %)	------	54 (91.52 %)
2.	Piperacillin/Tazobactam	30 µg	47 (79.66 %)	2 (3.38 %)	10 (16.94 %)
3.	Meropenem	10 µg	13 (22.03 %)	24 (40.67 %)	22 (37.28 %)
4.	Imipenem	10 µg	5 (8.47 %)	13 (22.03 %)	44 (74.57 %)
5.	Amikacin	30 µg	21 (35.59 %)	6 (10.16 %)	32 (54.23 %)
1.	Tigecycline	30 µg	46 (77.96 %)	------	13 (22.03 %)
2.	Ceftriaxone	30 µg	13 (22.03 %)	23 (38.98 %)	23 (38.98 %)
3.	Levofloxacin	5 µg	2 (3.38 %)	-------	57 (96.61 %)
4.	Nitrofurantoin	300 µg	5 (8.47 %)	11 (18.64 %)	43 (72.88 %)

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

DISCUSSION

Validator machines for tickets validation are fundamental parts of modern public transportation systems, serving as key junctions for fare collection and passenger validation (Ayare *et al.*, 2023). The implementation of efficient transit systems is essential for mitigating transportation challenges and improving overall mobility in densely populated urban areas, where traffic congestion and overcrowding are common (Busari *et al.*, 2021). These validator machines play a crucial role in simplifying the boarding process and ensuring fare compliance, thereby contributing to the efficiency and sustainability of urban transit networks (Hasan *et al.*, 2023). Despite their significance in enhancing the functionality of public transportation systems, validator machines are susceptible to surface contamination and microbial colonization, posing potential health risks to passengers and transit personnel (Chebon & Sonoiya. 2019).

In the current study, a total of 124 swab samples were collected from the in and out validator machines (4 validator machines in each station) across 31 BRT stations in Peshawar. Out of 124 samples, 65 (52.41 %) of the bacterial isolates were gram positive and 59 (47.58 %) of the isolates were gram negative. Similarly, Hernandez *et al.* (2020) isolated both gram-positive and gram negative bacterial isolates from Mexico City Metro. Bai *et al.* (2023) also reported airborne gram positive and gram negative bacterial isolates from public transit systems during covid pandemic. Similar to our study, Cave *et al.* (2021) also reported gram positive bacteria from railway and bus stations. Liaqat *et al.* (2023) reported bacterial presence in public transport vehicles and stations, such as buses and trains.

The current study identified *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Klebsiella* and *Proteus* species. Arasavilli *et al.* (2024) identified *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus* from Kharagpur railway station. Similarly, according to Hernandez *et al.* (2020), Mexico City Metro was dominated by the phylum *Actinobacteria* and *Streptococcus* (9%), *Staphylococcus* (5%), (*S. epidermidis*; 4%) *Cutibacterium* (15%) (*C. acnes* 13%) and *Corynebacterium* (13%). Similarly, Singh *et al.* (2022) reported *Klebsiella*, *Enterococci*, *Staphylococci* and *Acinetobacter* species from railway and bus station.

In the current study, 22 (33.84 %) of the *S. aureus* isolates were identified as methicillin resistant *S. aureus* (MRSA). Similar to our study, Cave *et al.* (2021) isolated CA-MRSA (26 %).

Thiruvengadam *et al.* (2020) isolated MRSA from Chennai. Similarly, Gu *et al.* (2020) reported

3.4 % of MRSA from shared bicycles in China. Several other researchers, including Bai *et al.* (2023), Khoothiam *et al.* (2023) and Kansaen *et al.* (2023) reported MRSA isolates from public transport areas.

In the current study, the antibiotic suscpetibility testing was performed for a total of 59 gram negative bacterial isolates. All the gram negative isolates were MDR, showing resistance to multiple drugs. Piperacillin/Tazobactam was the most sensitive antibiotic against the tested species. Similar to the current study, Wang *et al.* (2020) isolated gram negative bacteria from public transport system. Taha *et al.* (2023) also reported multi drug resistant bacterial isolates from self-service facilities in Sakaka city, Saudi Arabia. Similarly, Kaur *et al.* (2020) isolated carbapenem

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

resistant MDR gram negative bacteria isolates from bus stands and railway stations.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The study conducted antibiotic susceptibility testing on both gram-positive and gram-negative bacterial isolates from BRT stations in Peshawar. Results revealed a concerning prevalence of antibiotic resistance, notably with multi-drug resistance (MDR) among gram-negative bacteria isolates. Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified, indicating a potential public health threat. However, vancomycin and linezolid remained effective against most of the gram-positive bacterial isolates. Piperacillin/Tazobactam showed promising efficacy against gram-negative strains.

REFERENCES

Alazmi, B. M. (2023). High Bacterial Contamination Load of Self-Service Facilities in Sakaka City, Aljouf, Saudi Arabia, with Reduced Sensitivity to Some Antimicrobials. *Microorganisms*, *11*(12), 2937.

Siriphap, A. (2023). Prevalence, antimicrobial resistance, and enterotoxin gene profiles of Staphylococcus aureus isolated from mobile phones of the food vendors in Phayao province, Thailand. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), 68.

Abaitua Knight, A. M. (2023). Work on track!: A train interior design concept to meet contemporary work trends and needs.

Ainsalu, J., Arffman, V., Bellone, M., Ellner, M., Haapamäki, T., Haavisto, N., & Åman, M. (2018). State of the art of automated buses. *Sustainability*, *10*(9), 3118.

Ak, M. F., & Demir, E. (2023). A novel qualitative risk assessment using the interval-valued spherical fuzzy extension of TOPSIS method: a case study in rail transit systems. *Neural Computing and Applications*, 1-24.

Al-Ezzy, A. I. A., Khazzal, S. A., & Qasim, A. R. (2021). Multidrug Resistant Behavior of P Mirabilis Isolated From patients Urinary Tract Infections. *Diyala J. Vet. SciPrint*. ISSN: 2410-8863, 1(3), 23-37.

Ampu Harikrishnan, M. S. K., & Kumari, S. A Study on the Effect of Organizational Culture on Performance Management and Competency Development in a Private University in Himachal Pradesh. *International Journal of Research and Analytical*, *76*(12), 1-6.

Apata, O., Bokoro, P. N., & Sharma, G. (2023). The risks and challenges of electric vehicle integration into smart cities. *Energies*, *16*(14), 5274.

Arasavilli, S., Taksal, P. A., Das, B. K., Chowdhury, S., & Bhattacharya, J. (2024). Green graphene supported Cu2O-Ag2O S-scheme photocatalyst with high disinfection activity for prevention of urinary tract infection in toilets: A metagenomic analysis. *Journal of Environmental Chemical Engineering*, 112703.

Arjyal, C., J. Kc and S. Neupane. 2020. Prevalence of methicillin-resistant *Staphylococcus aureus*

Arora, M., Tuchen, S., Nazemi, M., & Blessing, L. 2021. Transportation Research Interdisciplinary Perspectives. 11(21): 1-13.

Arowan, S. M. U. J., Das, K. K., & Feroz, F. (2021). Microbiological surveillance of air and contact surface of pubic transports with its correlation to human infection risks. *Stamford Journal of Microbiology*, *11*(1), 7-10.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Arunwuttipong, A., Jangtawee, P., Vchirawongkwin, V., Kangwansupamonkon, W., Asavanant, K., & Ekgasit, S. (2021). Public Buses Decontamination by Automated Hydrogen Peroxide Aerosolization System. *Open Access Macedonian Journal of Medical Sciences*, *9*(E), 847-856.

Ayare, Y., Lohiya, A. D. H., Gulhane, S., & Kumar, S. 2023. Designing an Electronic Ticketing System for Local Commuter Transportation. In *International Conference on ICT for Sustainable Development*, 1(11):. 577-592.

Bai, H., He, L. Y., Gao, F. Z., Wu, D. L., Yao, K. S., Zhang, M., & Ying, G. G. (2023). Airborne antibiotic resistome and human health risk in railway stations during COVID-19 pandemic. *Environment International*, *172*, 107784.

Bhalla, G. S., M. S. Sarao, D. Kalra, K. Bandyopadhyay and A. R. John. 2018. Methods of phenotypic identification of non-tuberculosis mycobacteria. *Pract. Lab. Med*, 12(18): 1-10.

Bhardwaj, L. K., Rath, P., Bajpai, S., Upadhaye, D., Jain, H., Kumar, N., & Sinha, S. 2023.

Bharti, B., Li, H., Ren, Z., Zhu, R., & Zhu, Z. (2022). Recent advances in sterilization and disinfection technology: A review. *Chemosphere*, 136404.

Bibri, S. E., & Krogstie, J. (2020). The emerging data–driven Smart City and its innovative applied solutions for sustainability: The cases of London and Barcelona. *Energy Informatics*, *3*, 1-42.

Budzynski, M., Luczkiewicz, A., & Szmaglinski, J. 2021. Assessing the risk in urban public transport for epidemiologic factors. *Energies*, *14*(15), 4513.

Busari, A. A., Loto, R. T., Ajayi, S. O., Odunlami, O., Folake, A., Kehinde, O., & Olawuyi, O. 2021. Ameliorating urban traffic congestion for sustainable transportation. In *IOP Conference Series: Materials Science and Engineering*, 1107(1): 1-10.

Caggiano, G., Apollonio, F., Triggiano, F., Diella, G., Stefanizzi, P., Lopuzzo, M., & Montagna,

Cave, R., Cole, J., & Mkrtchyan, H. V. 2021. Surveillance and prevalence of antimicrobial resistant bacteria from public settings within urban built environments: Challenges and opportunities for hygiene and infection control. *Environment international*, *157*(1):1-18.

Chebon, S., & Sonoiya, J. C. 2019. Diversity and Antibiotics Susceptibility of Bacterial Species on Hand Surfaces in Public Buses Plying Kenyatta National Hospital Route 7c in Nairobi. *Microbiology Research Journal International*, *27*(2), 1-9.

Conference on Indoor Environmental Quality (pp. 57-67). Singapore: Springer Nature Singapore.

COVID-19 and the Interplay with Antibacterial Drug Resistance.

Danko, D., Bezdan, D., Afshin, E. E., Ahsanuddin, S., Bhattacharya, C., Butler, D. J., & Black, C. 2021. A global metagenomic map of urban microbiomes and antimicrobial resistance. *Cell*, *184*(13), 3376-3393.

Eze, E. C., M. E. El Zowalaty and M. Pillay. 2021. Antibiotic resistance and biofilm formation of Acinetobacter baumannii isolated from highrisk effluent water in tertiary hospitals in South Africa. *J. Glob. Antimicrob. Resist*, 27(1): 82-90.

Fernandez-Iriarte, A., Duchaine, C., Degois, J., Mbareche, H., Veillette, M., Moreno,

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

N., & Moreno, T. (2021). Bioaerosols in public and tourist buses. *Aerobiologia*, *37*, 525- 541.

Force, R. T. (2020). Cleaning and Disinfecting Transit Vehicles and Facilities During a Contagious Virus Pandemic. 1(11): 1-46.

Golub, A., MacArthur, J., Brown, A., & Brakewood, C. 2021. Applying an equity lens to automated payment solutions for public transportation.

Gu, J., Xie, X. J., Liu, J. X., Shui, J. R., Zhang, H. Y., Feng, G. Y., & Zhang, A. Y. (2020).

Hasan, M., Al Afnan, S., & Arafat, A. 2023. *Design and Implementation of Online Ticketing System for Metro Rail* (Doctoral dissertation, Sonargaon University (SU).

Hernandez, A. M., Vargas-Robles, D., Alcaraz, L. D., & Peimbert, M. (2020). Station and train surface microbiomes of Mexico City's metro (subway/underground). *Scientific Reports*, *10*(1), 8798.

Hui, C. X., Dan, G., Alamri, S., & Toghraie, D. (2023). Greening smart cities: An investigation of the integration of urban natural resources and smart city technologies for promoting environmental sustainability. *Sustainable Cities and Society*, *99*, 104985.

in shrines. *Int. J. Microbiol*, 2020(2): 1-10.

Inya, O. J., Eneyi, E. E., & Agbor, O. J. (2022). Isolation of Antibiotic Resistant Bacteria from Abattoir Waste Water: A Case Study of Makurdi Metropolis. *J. Environ. Issues Clim. Change.*, 1(1): 11-17.

Javan, K., Altaee, A., BaniHashemi, S., Darestani, M., Zhou, J., & Pignatta, G. (2023). A review of interconnected challenges in the water–energy–food nexus: Urban pollution perspective towards sustainable development. *Science of The Total Environment*, 169319.

Kabanangi, F., Nkuwi, E. J., Manyahi, J., Moyo, S., & Majigo, M. (2021). High level of multidrug- resistant gram-negative pathogens causing burn wound infections in hospitalized children in dares salaam, tanzania. *Int. J. Microbiol.*, 20(21): 1-19.

Kalb, L., Babler, P., Schneider-Brachert, W., & Eckl, D. B. (2022). Antimicrobial Photodynamic Coatings Reduce the Microbial Burden on Environmental Surfaces in Public Transportation—A Field Study in Buses. *International journal of environmental research and public health*, *19*(4), 2325.

Kansaen, R., Boueroy, P., Hatrongjit, R., Kamjumphol, W., Kerdsin, A., & Chopjitt, P. (2023). The Occurrence and Characteristics of Methicillin-Resistant Staphylococcal Isolates from Foods and Containers. *Antibiotics*, *12*(8), 1287.

Kaur, R., Singh, D., Kesavan, A. K., & Kaur, R. (2020). Molecular characterization and antimicrobial susceptibility of bacterial isolates present in tap water of public toilets. *International Health*, *12*(5), 472-483.

Khoothiam, K., Prapasawat, W., Yosboonruang, A., Rawangkan, A., Phuangsri, C., Rupprom, K.,

Kim, J., Han, S. J., & Yoo, K. (2022). Dust-Associated Bacterial and Fungal Communities in Indoor Multiple-Use and Public Transportation Facilities. *Atmosphere*, *13*(9), 1373.

Klimenko, N. S., Tyakht, A. V., Toshchakov, S. V., Shevchenko, M. A., Korzhenkov, A. A., Afshinnekoo, E., & Alexeev, D. G. 2020. Co-occurrence patterns of

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

bacteria within microbiome of Moscow subway. *Computational and Structural Biotechnology Journal*, *18*, 314-322.

Lanzini, P., Nguyen, T. T., & Slanzi, D. (2024). The Role of Market Insights in Shaping Sustainable Mobility in Fast Developing Countries: The Case of Vietnam. *Sustainability*, *16*(2), 651.

Liaqat, I., Zafar, F., & Zubair, H. A. (2023). A review on prevalence of viral and bacterial load on public transport. *Pure and Applied Biology (PAB)*, *13*(1), 22-37.

Lu, K., Liu, J., Zhou, X., & Han, B. 2020. A review of big data applications in urban transit systems. *IEEE Transactions on Intelligent Transportation Systems*, *22*(5): 2535-2552.

M. T. (2021). SARS-CoV-2 and public transport in Italy. *International Journal of Environmental Research and Public Health*, *18*(21), 11415.

Meyer, M. D., & Elrahman, O. A. (2019). *Transportation and public health: An integrated approach to policy, planning, and implementation*. Elsevier.

Moh, Y. (2017). Solid waste management transformation and future challenges of source separation and recycling practice in Malaysia. *Resources, Conservation and Recycling*, *116*, 1-14.

Mohammed, M. H., Farghaly, R. M., & Abdel-Aziz, N. M. (2023). The effect of some essential oils against biofilm producing *Pseudomonas aeruginosa* of meat sources. *Int. J. Veter. Sci.*, 6(1): 100-115.

Moreno, T., Pintó, R. M., Bosch, A., Moreno, N., Alastuey, A., Minguillón, M. C., & Querol, X. (2021). Tracing surface and airborne SARS-CoV-2 RNA inside public buses and subway trains. *Environment international*, *147*, 106326.

Mughal, A. A. (2019). Cybersecurity Hygiene in the Era of Internet of Things (IoT): Best Practices and Challenges. *Applied Research in Artificial Intelligence and Cloud Computing*, *2*(1), 1-31.

Nicolaides, C., Avraam, D., Cueto-Felgueroso, L., González, M. C., & Juanes, R. (2020). Hand- hygiene mitigation strategies against global disease spreading through the air transportation network. *Risk Analysis*, *40*(4), 723-740.

Njoku, A. U., & Joseph, M. 2022. Covid-19 and Environmental Racism. In *Handbook of Social Sciences and Global Public Health* (pp. 1-19). Cham: Springer International Publishing.

Obregón, S., & Rodríguez-González, V. (2021). Photocatalytic TiO 2 thin films and coatings prepared by sol–gel processing: A brief review. *Journal of Sol-Gel Science and Technology*, 1-17.

Omari, R., Zotor, F., Baah-Tuahene, S., & Arthur, W. (2022). Handwashing knowledge, attitudes, and practices in Ghana. *Journal of Preventive Medicine and Hygiene*, *63*(1), E59.

Oxford University Press, USA.

Passi, A., Nagendra, S. S., & Maiya, M. P. (2021). Assessment of exposure to airborne aerosol and bio-aerosol particles and their deposition in the respiratory tract of subway metro passengers and workers. *Atmospheric Pollution Research*, *12*(11), 101218.

Passi, A., Nagendra, S. S., & Maiya, M. P. (2023, February). Investigation of Indoor Air Quality and Passenger's Exposure in Underground Rapid Transit System. In *Asian*

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Prats, E. V. (2020). *WHO guidance for climate-resilient and environmentally sustainable health care facilities*. World Health Organization. *23*(3): 1-45.

Prevalence and transmission of antimicrobial-resistant Staphylococci and Enterococci from shared bicycles in Chengdu, China. *Science of The Total Environment*, *738*, 139735.

Redhwan, A., Sonbol, H., Al Tami, M., Alotaibi, S., Alzahrani, S., ALanazi, M., & Ameen, F. (2021). Molecular identification of airborne bacteria from metro stations in Riyadh. *Fresenius Environmental Bulletin*, *30*(1), 431-440.

Sidorchuk, R., Skorobogatykh, I., Lukina, A., Mkhitaryan, S., & Stukalova, A. (2020). Access to the rail station as a customer value: Simulation of passenger flows in rail stations with disinfection gateway installations. *Journal of Open Innovation: Technology, Market, and Complexity*, *6*(4), 122.

Singh, B. R., Karthikeyan, R., Sinha, D. K., Vinodhkumar, O. R., Jaykumar, V., Yadav, A., & Agri, H. (2022). Potentially Pathogenic Bacteria in Water Bodies and Drinking Water Supplies in and Around Bareilly, India. *Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)*, *5*(9).

Stanley, H. O. (2022). Antimicrobial analysis of isolates from sea food exposed to different preservation techniques. *GSC Biol. Pharm. Sci.*, 18(2): 15-20.

Taha, A. E., Alduraywish, A. S., Alanazi, A. A., Alruwaili, A. H., Alruwaili, A. L., Alrais, M. M.,

Taylor, B. D., & Ding, H. (2021). Making transit safe to ride during a pandemic: what are the risks and what can be done in response. 1(9): 1-11.

Tennakoon, V., Wiles, J., Peiris-John, R., Wickremasinghe, R., Kool, B., & Ameratunga, S. (2020). Transport equity in Sri Lanka: Experiences linked to disability and older age. *Journal of Transport & Health*, *18*, 100913.

Thiruvengadam, S., Moganalaxmi, R., Narmadha, V., Sowndariya, T., & Romauld, S. I. (2020). Incidence, Prevelance and Management of Methicillin-Resistant Staphylococcus aureus in Chennai. *Research Journal of Pharmacy and Technology*, *13*(2), 815-818.

Tirachini, A., & Cats, O. (2020). COVID-19 and public transportation: Current assessment, prospects, and research needs. *Journal of public transportation*, *22*(1), 1-21.

Wali, N., Shabbir, A., Wajid, N., Abbas, N., & Naqvi, S. Z. H. (2022). Synergistic efficacy of colistin and silver nanoparticles impregnated human amniotic membrane in a burn wound infected rat model. *Scient. Rep.*, 12(1): 1-12.

Wang, J., Yuan, Y., Xu, J., Wu, Y., He, M., Zhou, R., & Shen, Q. (2020). Microbial Community of the Public Transport System Revealed the Potential Risk of Pathogen Transmission: A Pilot Research. *Investigación Clínica*, *61*(2), 935-942.

Wen, Y., Leng, J., Shen, X., Han, G., Sun, L., & Yu, F. 2020. Environmental and health effects of ventilation in subway stations: a literature review. *International journal of environmental research and public health*, *17*(3), 1084.

White, F., Stallones, L., & Last, J. M. (2013). *Global public health: Ecological foundations*.

Zahraei Ahmadabadi, S. M., & HOUSHMANDFARZANEH, S. (2023). Ramsar Maritime Terminal: a public space in a moderate coastal zone.

Zhang, W. H., Yuan, Q., & Cai, H. (2023). Unravelling urban governance challenges:

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Objective assessment and expert insights on livability in Longgang District, Shenzhen. *Ecological Indicators*, *155*, 110989.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

CHAPTER 8
APPENDICES



App. 1. Staphyloccus colonies



App. 2. Acinetobacter colonies



App. 3. Pseudomonas colonies

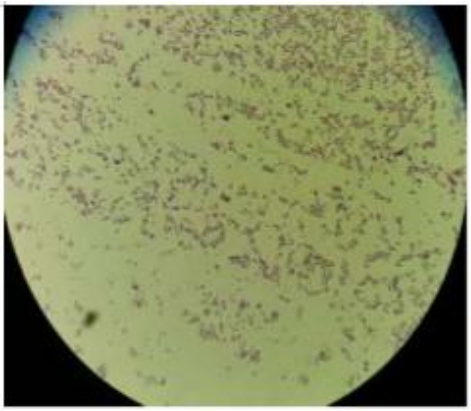


App. 4. Klebsiella colonies

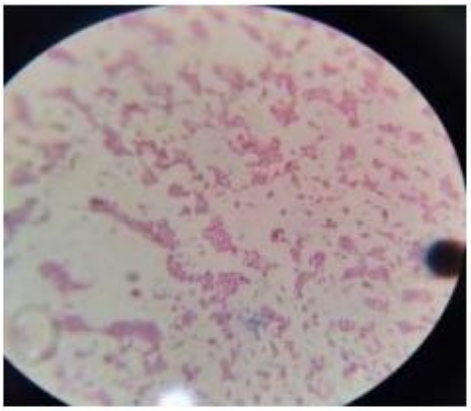
Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about



App. 5. Gram Positive bacteria



App.6. Gram negative bacteria



App. 8.7. Biochemical Tests

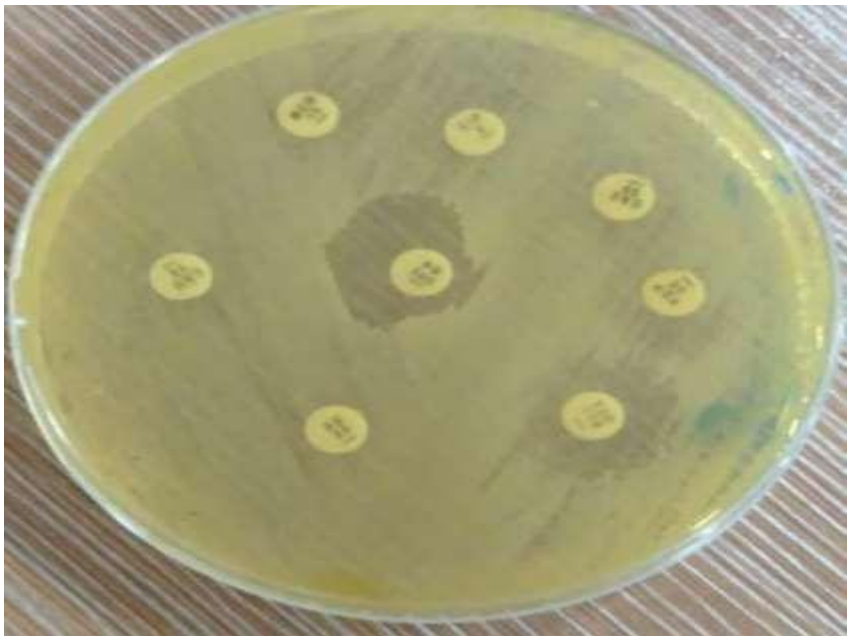
Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about



App. 8. Catalase Test



App. 9. Antibiotic susceptibility testing