Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Prevalence, antibiotic susceptibility and plasmid profile of bacteria isolated from door handles of washrooms of a hospital in Bhopal, Madhya Pradesh

Shipra Shrivastava [1,*], **Naveen Khare** [2]

[1] *Dept. of Biochemistry, NIMS Institute of Allied Medical Sciences, Jaipur, Rajasthan, India*
[2] *Dept. of Biochemistry, Teva Pharmaceutical, Bengaluru, Karnataka, India*

ARTICLE INFO

Article history:
Received 09-05-2023
Accepted 22-06-2023
Available online 18-07-2023

Keywords:
Door handle
Gram staining
Antibiotics
Susceptibility

ABSTRACT

Background: This study was conducted to determine the bacterial contamination in door handles of washrooms of a hospital, to determine the prevalence and antibiotic susceptibility pattern of the isolates. Plasmid profile was done to observe the presence or absence of plasmid among isolated bacteria from door handles of washrooms of a hospital.

Materials and Methods: Washrooms of four different wards of a hospital were included in this study. Manual of Systematic Bacteriology and antibiotic susceptibility test was done by Kirby-Bauer method. Plasmid extraction was done according to modified hot alkaline method and staining technique and biochemixcal testing.

Results: Out of the 16 samples processed, 16 (100%) of them showed bacterial growth. A total of forty three (43) isolates were obtained. The bacteria isolated were *Staphylococcus* spp. (37.21%), *Bacillus* spp.(18.6%), *E. coli*.(16.28%), Fecal Coliform (13.95%), Micrococcus spp.(6.98%), *Pseudomonas* spp.(4.65%), *Klebsiella* spp.(2.33%).Plasmid profiling revealed (11 out of 43) bacterial isolates contained 1 or more plasmids with different profiles.. Among the 43 isolates, 83.72% were found resistant to more than two antibiotics. Highest resistance percentage of the isolates was observed against Penicillin G (95.35%) followed by SXT (74.42%) and amoxicillin (65.12%), rifampicin (55.81%), tetracycline (18.60%), ciprofloxacin (23.26%), chloramphenicol (4.65%), gentamycin, (2.33%) and streptomycin (6.98%).

Conclusion: Findings of this study indicate the presence of bacterial strains resistant to more than two antibiotics in door handles of washrooms of a hospital which can serve as potential source of diseases.

1. Introduction

One of the major causes of HAIs that is associated with patient morbidity and mortality is fomites.[1,2] In hospitals, fomites can serve as a reservoir of pathogens being spread from the inanimate environment to an animate (patient) environment via the hands of health care workers (HCW).[3–5] To reduce morbidity and mortality in hospitals, identification of common fomites associated pathogens in any hospital settings is important. Because, the most important factor in prevention of a disease is to simply identify what has been transferring the disease.

Among the vast range of fomites, door handles is one of the most common one, which serves as route for contamination. Hard and nonporous surface allows more adhesion of bacteria to it. Due to having such a surface, door handles provide the highest rate of bacterial transfer to the hands.[6]

Bacterial pathogens that have been isolated from door handles in previous studies includes S. aureus,

* *Corresponding author*.
E-mail address: shipra2853@gmail.com (S. Shrivastava).

https://doi.org/10.18231/j.ijmmtd.2023.016

K.pneumonia, *E. coli*, *Enterobacter* spp, *Citrobacter* spp, *P. aeruginosa*, *Proteus* spp, *Streptococcus* spp, *Salmonella* spp, *Shigella* spp, *Campylobacter* spp.[7,8] These organisms have been known to cause one or more diseases that are mild and could be sometimes serious. The examples of such diseases range from simple skin diseases like pimple, impetigo, scalded skin syndrome to respiratory diseases like, pneumonia to even severe meningitis, osteomyelitis, rhinoscleroma, kidney failure, septicemia and so on.[9]

Toilets act as a vehicle for the transmission of pathogens from gut, respiratory tract and skin via hands and surfaces from one person to another. Later, when another person holds that door handle, the bacteria can pass on. In adverse situation, such a transfer may even bring about outbreaks of infection.[10]

The hands serve as a medium for the propagation of microorganisms from place to place and from person to person. Although, it is nearly impossible for the hand to be free of microorganisms, the presence of pathogenic bacteria may lead to chronic or acute illness. Human hands usually harbor microorganisms both as part of the body normal flora as well as transient microbes collected from the environment.[11]

The occurrence of this may be attributed to the unhygienic use of the toilet facilities, which results to the filthy contamination of the place including door-handle, which individuals are less likely to see as contaminated.[12] Improper use of the toilets, inadequate cleanliness of the toilets facilitates transmission of bacteria from the toilets to even household living rooms. Contaminated hands of toilet users can transmit the bacteria from their hands to the flushing handles, door handles and faucets of the toilets as well as household door handles and equipment. Toilet flushing results in a large quantity of flush aerosols, which can reach the seats and leads, surrounding floors and nearby surfaces. The ability of the pathogen deposited to survive on the different surfaces in the toilets poses a great risk of infection to the toilet users.[13] The time of survival depends on the type of pathogen, majority including Shigella species, *Escherichia* species, *Clostridium* species, severe acute respiratory syndrome (SARS) coronavirus, and norovirus which can survive on surfaces for weeks or even months.[5]

2. Aims and Objectives

The aim of identify and evaluate the occurrence of bacterial contaminations form the door handles of washrooms of a hospital and their harmful consequence to public health.

Overall the main objectives were: To determine the presence of bacterial contamination, to isolate and identify both Gram-positive and Gram-negative bacteria, to determine the prevalence and antibiotic susceptibility pattern of bacteria isolated from washroom door handles of hospital.

3. Materials and methods

3.1. Study area

The study was carried out in the, Laboratory of the Department of Medical Lab Technology (Microbiology) of Rajeev Gandhi College and General Hospital, Bhopal.

3.2. Sample size

A total of 16 door handles of washrooms of Rajeev Gandhi College and General Hospital, Bhopal were included in this study.

3.3. Sample collection

The samples were collected from toilet door handles using the swab-rinse method. Door knobs/ handles were swabbed with sterile cotton swabs moistened with sterile normal saline. The swab was wiped firmly on the entire surface of the door handles/ knobs. It was then introduced into a test-tube containing sterile nutrient broth. Then it was immediately transported to the Microbiology Research Laboratory of Rajeev Gandhi College & General Hospital Bhopal for further processing and analysis. The test tube containing the sample incubated at 37ºC overnight.

3.4. Technique

After 24 hours, each sample was streaked onto Nutrient agar, MacConkey agar, Mannitol salt agar and Membrane fecal coliform agar plates. Four-quadrant streak plate technique was performed. All the plates were incubated for 24 hours at 37ºC. After the overnight incubation, the plates were observed for colony characteristics. Isolated colonies were then sub-cultured onto fresh nutrient agar. Single isolated colonies from nutrient agar plates were subjected to Gram staining, Spore staining and Standard Biochemical tests to identify the organism. Media used for biochemical tests are: Indole broth, Methyl Red (MR) broth, Voges-Proskauer (VP) broth, Simmons citrate agar, Triple Sugar Iron (TSI) agar, Motility Indole Urease (MIU) agar Nitrate reduction broth.

Plasmid extraction of the isolates were done according to the modified hot alkaline method by Kado and Liu.Modified hot alkaline method by Kado and Liu (Kado and Liu, 1981), Agarose gel electrophoresis.

4. Results

After performing the biochemical tests and observing cultural and morphological characteristics 43 isolates were identified from 16 different samples collected from hospital toilet doorknobs. The isolates include *Staphylococcus* spp., (found in 16 samples), *Bacillus* spp., (found in 8 samples), *E.coli* (found in 7 samples), Fecal coliform (found in 6 samples), Micrococcus spp., (found in 3 samples),

Pseudomonas spp, (found in 2 samples), Klebsiella spp. (found in 1 sample).

Table 1: Prevalence of bacteria isolated from door handles of washrooms of a hospital

Name of the Bacteria	Number of isolates	Percentage %
Staphylococcus spp.	16	37.21
Bacillus spp.	8	18.6
E. coli.	7	16.28
Fecal Coliform	6	13.95
Micrococcus spp.	3	6.98
Pseudomonas spp.	2	4.65
Klebsiella spp.	1	2.33
	Total= 43	100

Table 2: Distribution of the isolates according to Gram's Reaction

Isolates	Number of isolates	Percentage (%)
Gram Positive	27 (out of 43)	62.8%
Gram Negative	16 (out of 43)	37.2%

Table 3: Percentage of isolates resistant to antibiotics

Serial number	Antibiotics	Percentage of isolates resistant to antibiotics	No. of isolates resistant to antibiotics
1	Amoxicillin	65.12	28
2	Chloramphenico	4.65	2
3	Ciprofloxacin	23.26	10
4	SXT	74.42	32
5	Rifampicin	55.81	24
6	Gentamicin	2.33	1
7	Tetracycline	18.60	8
8	Streptomycin	6.98	3
9	Penicillin G	95.35	41

The most resistance was seen against penicillin G, with a number of 41(95.35%) isolates being resistant against it. Next to penicillin G, 32 isolates were resistant to SXT, giving a percentage of 65.12. The third highest resistance was seen against, amoxicillin where 32 (65.12) isolates were resistant to it. Whereas, the isolates were most sensitive toward gentamicin (2.33%) followed by resistance to chloramphenicol (4.65%) and streptomycin (6.98%).

5. Discussion

The result obtained from this study was that out of 16 samples 16 of them showed bacterial contamination. After conducting the biochemical tests, the isolates were confirmed as the following organisms: *E. coli*, *Klebsiella* spp., Micrococcus spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., and some other fecal organisms.

Table 4: Plasmid pattern and number of isolates hosting the plasmid

Pattern	Lane in Fig.	Approximate size of plasmid (MDa)	Number of isolates hosting the pattern
1	2,7,8	~85	3
2	4,5,6,10,11	~8	5
3	12,13	~50	2
4	15	~35.6	1
5	1,16	~85.0, ~4.8, ~3.7, ~3.4, ~2.0	1 (v517) control strain of *E.coli*

In this study, among the isolates, the most predominant bacteria were *Staphylococcus* spp., with a percentage of 37. This is anticipated as it is a major component of the normal flora of the skin and nostrils. The findings of other researchers.[8,11,13] is in accordance with this finding.

Staphylococcusspecies (54.7%) was the most frequent bacteria isolated in hospital environment. In contrast, the result of this study did not agree with the work of Orskov et al (2005) which showed that *Staphylococcus aureus* was the least isolated bacteria.[14]

As mentioned before, Gram-positive bacteria are found more in the hospital fomites than Gram Negative one. This can become dangerous as Gram positive bacteria are causing more infections than ever before in surgical patients, who are increasingly aged, ill and debilitated.[15]

Isolation of more Gram positive bacteria than Gram negative canbe explained, as they are members of the body flora of both asymptomatic carriers and sick persons. These organisms can be spread by the hand, expelled from the respiratory tract or transmitted by animate or inanimate objects.[16] Their main source(s) of colonization on the fomites might likely be nasal carriage by hospital personnel,[17] likely facilitated by hand-to-mouth or hand-to-nose contact while using these fomites, and/or by improper hand washing. Isolation of *Staphylococcus aureus* from almost all the fomites indicates their ubiquitous nature. Additionally, they can be sources of infection to patients as previously noted.[4,18,19]

A high percentage of *Bacillus* spp. was isolated from hospital washroom door handles. This is also in agreement with the research carried out by Brooks et al., (2007) who reported that *Bacillus* spp. was found to be the predominant organism among all the organisms that were isolated from door handles.

Bacillus spp., the only Gram positive bacilli encountered in this study, has been isolated with the highest frequency in some studies in Nigeria.[2,4] This organism forms endospores, which, allows them to settle well on the surface from fomites from air.

Although, Gram positive organisms were more frequently isolated in this study, the Gram negative bacterium *E. coli* was also isolated from toilet doorknobs.

This indicates improper hand washing after the use of toilet.

Pseudomonas spp., and *Klebsiella* spp., and some fecal coliform bacteria were also isolated which are Gram negative. Micrococcus spp., which is Gram positive bacteria, was also isolated from hospital toilet door knobs.

From the findings in this study, it was observed that most of the isolates obtained were resistant to most commonly used antibiotics. These antibiotics are Amoxicillin, SXT and Penicillin G. The resistance to these antibiotics that antibiotic resistant microorganism contaminates environmental surfaces such as toilet. Moreover, reported that most of the isolates obtained in their study were resistant to commonly used antibiotics such as Amoxicillin and Ampicillin.

The plasmid profile showed the absence of plasmids for maximum isolates. The occurrence of plasmids in Gram negative bacteria was found. Eleven isolates including *E.coli, Klebsiella* spp., and fecal coliforms contained plasmid. Findings also indicated that isolates that were resistant to more than two antibiotics may harbor plasmid. Isolation of plasmids using agarose gel electrophoresis and observation under UV trans-illuminator showed the bands for the *E.coli*, *Klebsiella* spp., and other fecal coliforms with the molecular weights of plasmids ranging from approximately 8 to 85 MDal.

6. Conclusion

The causes of these infections in hospitals can be connected to increased microbial load of fomites of these places. The data from this study indicates that there is a high level of bacterial contamination on door handles of hospital washrooms. In this study most of the isolates were resistant to more than two antibiotics and plasmids were found in some multi-drug resistant organisms. Further study can be done to find out any correlation between multidrug resistance of bacteria and presence of plasmids. So, further research upon plasmid will also be very significant.

7. Limitations

Other like different types of culture media used for identification and antibiotics used for determination of organisms.

8. Conflict of Interest

None.

9. Source of Funding

None.

References

1. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacterspecies. *Am J Infect Control*. 2010;38(5):25–33.
2. Nwankiti OO, Ndako JA, Nwankiti AJ, Okeke OI, Uzoechina AR, Agada GO, et al. Computer keyboard and mouse: etiologic agents for microbial infections. *Nat Sci*. 2012;10(10):162–6.
3. Bhalla A, Pultz NJ, Gries DM, Ray AJ, Eckstein EC, Aron DC, et al. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. *Infect Control Hosp Epidemiol*. 2004;25(2):164–7. doi:10.1086/502369.
4. Ikeh EI, Isamade ES. Bacterial flora of fomites in a Nigerian multi-disciplinary intensive care unit. *Lab Med*. 2011;42(7):411–3.
5. Kramer A, Scwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis*. 2006;6:130. doi:10.1186/1471-2334-6-130.
6. Rusin P, Maxwell S, &gerba C. Comparative surface to hand and fingertip to mouth transfer efficiency of gram positive bacteria, gram negative bacteria and phage. *J Appl Microbiol*. 2002;93(4):585–92.
7. Itah AY, Ben AE. Incidence of enteric bacteria and Staphylococcus aureus in day care centers in Akwa Ibom State, Nigeria. *Southeast Asian J Trop Med Public Health*. 2004;35(1):202–9.
8. Nworie A, Ayeni JA, Eze UA, O AS. Bacterial contamination of door handles/knobs in selected public conveniences in abuja metropolis, nigeria; a public health threat. *Continental J Med Res*. 2012;6(1):7–11.
9. Clauditz A, Resch A, Wieland KP, Peschel A, Gotz F. Staphyloxanthin plays a role in the fitness of Staphylococcus aureus and its ability to cope with oxidative stress. *Infect Immun*. 2006;74(8):4950–3. doi:10.1128/IAI.00204-06.
10. Maori L, Agbor VO, Ahmed, A W. The prevalence of bacterial organisms on toilet door handles in Secondary Schools in Bokkos L. G. A., Jos, Plateau Sate, Nigeria. *IOSR J Pharm Biol Sci*. 2013;8(4):85–91.
11. Dodrill L, Schmidt WP, Cobb E, Donachie P, Curtis V, De-Barra M, et al. The Effect of Hand washing with Water or Soap on Bacterial Contamination of Hands. *Int J Environ Res Public Health*. 2011;8(1):97–104. doi:10.3390/ijerph8010097.
12. Available from: http://ezineartides.com.
13. Boone SA, Gerba CP. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol*. 2007;73(6):1687–96. doi:10.1128/AEM.02051-06.
14. Orskov I, Orskov F, Jam B, Jann K. Serology, chemistry and genetics of O and K antigens of Escherichia coli. *Bacteriol Rev*. 1997;41(3):667–710. doi:10.1128/br.41.3.667-710.1977.
15. Barie PS. Antibiotic-resistant Gram-positive cocci: implications for surgical practice. *World J Surg*. 1998;22(2):118–26.
16. Chikere CB, Omoni VT, Chikere BO. Distribution of potential nosocomial pathogens in a hospital environment. *Afri J Biotech*. 2008;7(20):3535–8.
17. Graham P, Lin S, Larson EA. US population based survey of Staphylococcus aureuscolonization. *Ann Intern Med*. 2006;144(5):318–25.
18. Hartmann B, Benson M, Junger A, Quinzio L, Röhrig R, Fengler B, et al. Computer keyboard and mouse as a reservoir of pathogens in an intensive care unit. *J Clin Monit Comput*. 2004;18(1):7–12.
19. Inweregbu K, Dave J, Pittard A. Nosocomial infections. *Contin Educ Anaesth, Crit Care Pain*. 2005;5(1):4–17.

Author biography

Shipra Shrivastava, Research Scholar https://orcid.org/0009-0001-9418-1855

Naveen Khare, Analytical Research 2

Cite this article: Shrivastava S, Khare N. Prevalence, antibiotic susceptibility and plasmid profile of bacteria isolated from door handles of washrooms of a hospital in Bhopal, Madhya Pradesh. *IP Int J Med Microbiol Trop Dis* 2023;9(2):81-84.