European Journal of Applied Sciences 5 (3): 76-79, 2013
ISSN 2079-2077

DOI: 10.5829/idosi.ejas.2013.5.3.66188

Antibiotic Resistance Profile of Microbial Isolates of Toilet-Bowl of Some Students' Hostels in Ogbomoso, Nigeria

[1]A.G. Adewoyin, [2]O.N. Majolagbe, [1]T. Ogunmoyewa and [1]M.A. Abejide

[1]Department of Science Laboratory Technology,
[2]Department of Pure and Applied Biology (Microbiology Unit),
Ladoke Akintola University of Technology, P.M.B. 4000 Ogbomoso, Oyo State, Nigeria

Abstract: A total of fifteen bacterial isolates was characterized from toilet bowl of six prominent student hostels. The antibiotic resistance profiles of the isolates to some commonly used antibiotics and disinfectants were investigated. The highest and lowest total bacterial counts (TBC) were 33.90±4.23 x 10^7 and 9.00±1.80 x10^7 CFU/ml, respectively. Most of the bacterial isolates were enteric, suggesting faecal contamination of the cistern. Out of the fifteen bacteria isolates identified, the genus *Streptococcus* was highly dominant with highest prevalence (19.36%) observed by *Streptococcus faecium* and least (3.23%) by *S. pyogenes* and *S. zymogenes*. Antibiogram of the bacterial isolates showed that the highest and lowest resistances noticeable in *Pseudomonas aeruginosa a*nd *Streptococcus zymogenes* were 90 and 20% respectively. About 80% of all the isolates resisted augmentin while 26.67% resisted pefloxacin and chloramphenicol. In conclusion, proper sanitary habit, ensuring proper and good personal hygiene practices after using the toilet is a better option to minimize transmission of potential pathogens.

Key words: Antibiotic · Toilet-Bowl · Antibiotic Resistance Profile

INTRODUCTION

The toilet is a system for the disposal of body waste [1]. Toilets which are difficult to wash and clean help in transmission of infection [2]. Large number of bacteria and viruses when seeded into toilets may remain air borne after droplet is produced by flushing and consequently settle on surfaces throughout the bathroom. Sanitary conditions in public places have always been a major problem, especially bathroom and restrooms, splashes and aerosol spread from toilet bowl directed at openings in the human body (vaginal opening and the anus) and the toilet room area present a substantial risk for people to become infected [3]. Barker and Jones [4] investigated the level of aerosol formation and fallout within a toilet cubicle after flushing a toilet contaminated with indicator organisms and reported that although a single flush reduced the level of micro organisms in the toilet bowl water, large number of micro organisms persisted in the toilet bowl water which are disseminated into the air by further flushes. Improper cleaning of hand after using the toilet has exposed many people to varieties of disease during toilet flushing and especially during evacuation processes. These undesirable elements of human evacuations can contain germs: bacteria fungi, viruses that produce a substantial risk for people to become infected [3].

Transmission of intestinal parasites and enteropathogenic bacteria is affected directly or indirectly through objects contaminated with feces [5]. Several of these pathogenic bacteria are known to survive on surfaces for extended period of time [6-8]. In studies done in England, it was found that pathogenic intestinal organisms such as *Salmonella* in Berker and Bloomfield research occurs in one of five people each year [7]. This work highlighted the incidence and frequency of occurrence of micro organisms in some toilet bowls collected from students' hostels and also investigated the antibiotic resistant profile of the isolated micro organisms against some commonly used antibiotics and disinfectants.

Corresponding Author: O.N. Majolagbe, Department of Pure and Applied Biology (Microbiology Unit), Ladoke Akintola University of Technology, P.M.B. 4000 Ogbomoso, Oyo State, Nigeria. Tel: +2348038360759.

MATERIALS AND METHODS

Media Preparation: All media used were prepared according to the manufacturer's specifications and then sterilized by autoclaving at 121°C for 15minutes.

Sample Collection and Maintenance: Samples were collected from the toilet bowl after a single flush, stored in clean sterile screw-capped bottles and then transported to the laboratory for further analysis. All samples were maintained at 4°C immediately after collection.

Isolation and Characterization of Microorganisms: An aliquot of the sample was taken, serially diluted and plated on media using the rock plate method for bacterial isolation [7]. Total bacterial counts (TBC) of the dilutions were recorded. Pure cultures of the bacterial isolates were obtained by sub-culturing on nutrient agar, MacConkey agar and blood agar, incubated at 37°C for 24 hours. The isolated organisms obtained were subjected to biochemical characterization for identification Bergey's manual of determinative bacteriology.

Antibiotic Susceptibility Testing (AST): The antibiotic susceptibility test was carried out by agar disc-diffusion method against the test isolates. Pure cultures of the bacteria were sub-cultured on Nutrient Agra (NA). Paper disc loaded with different concentrations of the antibiotics were placed on the surface of the each of the cultured plate and incubated at 37°C for 24-36 hours after which the inhibition zones were measured. The following antibiotics were used: Septrin (Sep, 10µg), Sparfloxacin (Spf, 10µg), Ciprofloxacin (Cip, 5µg), Amoxycillin (Amx, 25µg), Augmentin (Aug, 30µg), Gentamycin (Gen, 10µg), Pefloxacin (Pef, 5µg), Ofloxacin (Ofl, 5µg), Streptomycin (Str, 10µg) and Chloramphenicol (Chl, 5 µg). Two commonly used toilet disinfectants such as Harpic and Izal were used to further investigate the antimicrobial resistance profile of the isolates.

RESULTS AND DISCUSSION

A total of fifteen bacteria were isolated from the toilet-bowl of student's hostels. Figure 1 shows the total bacterial count and number of bacterial isolates present in each sample. The highest and lowest total bacterial count (TBC) of 33.90±4.23 x 10^7 and 9.00±1.80 x10^7 CFU/ml were recorded in sewage samples H_2 and H_5 respectively (Fig. 1). This might be due to the low level of sanitation level ensured by the students in daily usage of the modern day toilet facilities. Our result is contrary to that of Barker and Jones [4] who suggested that high population of the microorganisms is expected to be reduced after the first flush. The persistent increase in the bacterial load count in each of the samples might be due to disposal of body waste from the large number of students residing in the hostels [1]. Besides the insufficient toilet facilities and over usage of the toilet could have also led to the drastic increase in total bacterial count recorded. Table 1 shows that most of the bacterial isolates were enteric, suggesting faecal contamination of the toilet bowl. This is in agreement with the investigation of Flores *et al.* [10] who reported the bio-geographical patterns exhibited by bacteria across surfaces within public rest rooms. In addition, in all the six samples assessed, the highest number of bacterial isolates was recorded in sample site H_2; the same is in coincidence with the highest TBC recorded in the same site as earlier mentioned before (Fig. 1).

Table 1: Percentage occurrence of bacterial isolates in the examined toilet sewage samples

Bacterial isolates	Percentage (%) occurrence in samples
Aerobacter aerogenes	9.68
Bacillus cereus	16.13
Enterococcus aerogenes	3.23
Escherichia coli	3.23
Klebsiella aerogenes	6.45
Micrococcus acidiophilus	9.68
Micrococcus luteus	3.23
Proteus vulgaris	6.45
Pseudomonas aeruginosa	3.23
Staphylococcus aureus	3.23
Streptococcus bovis	3.23
S. fecalis	6.45
S. faecium	19.36
S. pyogenes	3.23
S. zymogenes	3.23

Our report showed faecal contamination of the surfaces and out of the fifteen bacteria isolates recorded, the genus *Streptococcus* were highly dominant with highest prevalence (19.36%) seen in *Streptococcus faecium* and least (3.23%) in *S. pyogenes* and *S. zymogenes* (Table 1). This is likely due to the fact that *Streptococcus* spp. is a commensal found in the intestines of humans [11, 12]. *S. faecium* which occurred in all the samples is also known as *Enterococcus faecium* since 1984 due to re-categorization [13]. The ability of Gram negative species such as *Escherichia coli*, *Klebsiella aerogenes* and *Pseudomonas aeruginosa* to grow to substantial numbers in samples of toilet sewage have been demonstrated [14- 17]. According to Guthrie [17], there is the possibility that infection can arise from direct

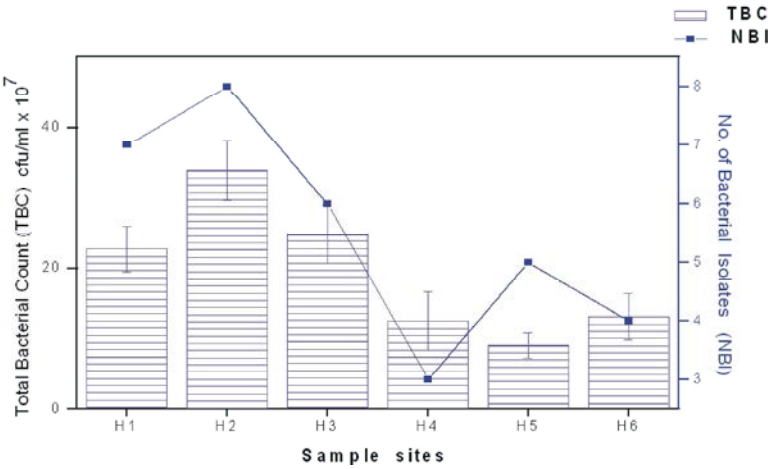
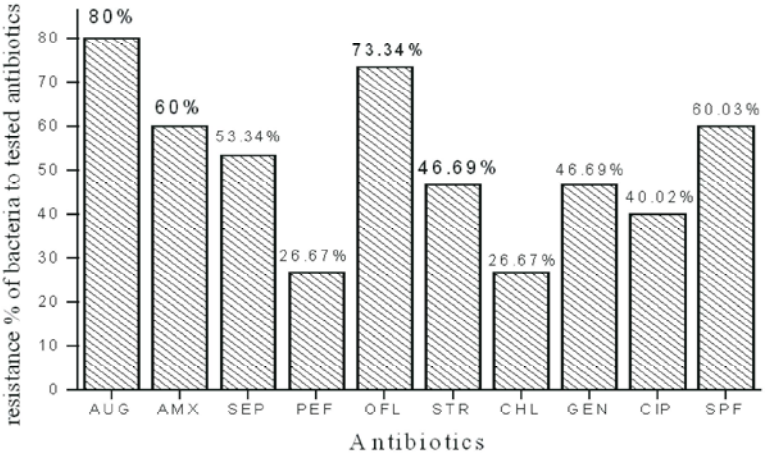


Fig. 1: Total bacterial count and number of bacterial isolates present in each sample site
TBC= total bacterial count; NBI= number of bacterial isolates



Sep– Septrin; Spf– Sparfloxacin; Cip– Ciprofloxacin; Amx- Amoxacilin; Aug– Augmentin; Gen– Gentamycin; Pef– Pefloxacin; Ofl– Ofloxacin; Str– Streptomycin; Chl- Chloramphenicol

Fig. 2: Resistance percentages of isolated bacteria to tested antibiotics

Table 2: Antibiogram of bacterial isolates

Bacterial isolate	No. of resisted antibiotic	% resistance
Streptococcus fecalis	Nd (0)	0
Streptococcus zymogenes	Aug, Amx (2)	20
Klebsiella aerogenes	Sep, Pef, Ofl, Str (4)	40
Aerobacter aerogenes	Sep, Chl, Gen, Str (4)	40
Proteus vulgaricus	Sep, Cip, Aug, Str (4)	40
Streptococcus faecium	Aug, Spf, Amx, Ofl (4)	40
Staphylococcus aureus	Aug, Spf, Amx, Ofl (4)	40
Streptococcus bovis	Aug, Spf, Amx, Ofl, Sep (5)	50
Micrococcus acidiophilus	Gen, Aug, Spf, Amx, Ofl (5)	50
Escherichia coli	Sep, Aug, Gen,Pef, Ofl, Str (5)	60
Micrococcus luteus	Gen, Aug, Spf, Amx, Ofl, Cip (6)	60
Enterobacter aerogenes	Spf, Cip, Aug, Gen, Pef, Ofl, Str (7)	70
Bacillus cereus	Pef, Aug, Spf, Amx, Ofl, Cip, Sep, Chl (8)	80
Streptococcus pyogenes	Gen, Aug, Spf, Amx, Ofl, Cip, Str, Sep, Chl (9)	90
Pseudomonas aeruginosa	Sep., Chl., Spf., Cip., Amx., Aug., Gen., Ofl., Str (9)	90

Sep– Septrin; Spf– Sparfloxacin; Cip– Ciprofloxacin; Amx- Amoxacilin; Aug– Augmentin; Gen– Gentamycin; Pef– Pefloxacin; Ofl– Ofloxacin; Str– Streptomycin; Chl- Chloramphenicol; Nd- not determined

contact with contaminated surfaces or by person-to-person or via the faecal-oral route. Table 2 shows the antibiogram of the bacterial isolates in which the highest (90%) were recorded in *Streprococcus pyogenes* and *Pseudomonas aeruginosa* while the lowest (20%) resistance was seen in *Streptococcus zymogenes*. Five out of the fifteen bacteria showed 40% resistance to the tested antibiotics and these include: *K. aerogenes*, *A. aerogenes, P. vulgaricus, S. faecium and S. aureus*. *S. bovis* and *M. acidiophilus* showed 50% resistance; *E. coli* and *M. luteus* showed 60% resistance; while only *E. aerogenes* and *B. cereus* showed 70% and 80% resistances respectively as indicated in Table 2. Figure 2 shows the resistance percentages of isolated bacteria to tested antibiotics with augmentin (80%), ofloxacin (73.34%), sparfloxacin (60.03%), amoxacillin (60%), septrin (53.34%), streptomycin (46.69%), gentamycin (46.69%), ciprofloxacin (40.02%), pefloxacin (26.67%) and chloramphenicol (26.67%). It has been reported earlier that some enterococci are intrinsically resistant to β-lactam based antibiotics and as well as many amino glycosides [11]. Finally, all the micro-organisms isolated from the toilet bowl water showed some level of susceptibility to Harpic and Izal toilet disinfectants, such disinfectants can be used to prevent the accumulation of such potential pathogens in toilets, thereby reducing the spread and transmission of infections.

Considering the antibiotic resistance of some of these micro organisms to some of the tested antibiotics it can be deduced that proper sanitary habit and hygiene practices after using toilets is a better option to minimize transmission of potential human pathogens especially in dense population where people reside.

REFERENCES

1. Cai, D. and M. You, 1998. An ergonomic approach to public squatting-type toilet design. Appl. Ergon., 29: 147-153.
2. Farrell, G., 1980. Toilet hygiene: clean round the bend. Health Soc Serv. J., 90: 1265.
3. Greed, C., 2006. "The role of the public toilet; pathogen transmitter or health facilitator?," Building serv. Eng. Res. Technol., 27(2): 1 -13.
4. Barker, J. and M.V. Jones, 2005. The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. J. Applied Microbiology, 99: 339-347.
5. World Health Organization, 1987. Prevention and control of intestinal parasitic infections. Geneva: World Health Organization, pp: 7-18. (Technical Report Series No. 749).
6. Noskin, G.A., V. Stosor, I. Cooper and L.R. Peterson, 1995. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infection Control and Hospital Epidemiology: The Official Journal of the Society of Hospital Epidemiologists of America, 16: 577-581.
7. Barker, J. and S.F. Bloomfield, 2000. Survival of Salmonella in bathroom and toilets in Domestic homes following salmonellosis". Journal of Applied Microbiology, 89: 137-144.
8. Bures, S., J.T. Fishbain, C.F. Uyehara, J.M. Parker and B.W. Berg, 2000. Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit. American Journal of Infection Control., 28: 465-471.
9. Wheeler, J.G., D. Sethi, J.M. Cowden, P.G. Wall, L.C. Rodrigues, D.S. Tompkins, D.S. Hudson and P.J. Roderick, 1999. Study of infectious intestinal disease in England: rates in the community, presenting to general practice and reported to national surveillance. British Medical Journal, 318: 1046-1055.
10. Flores, G. E., S. T. Bates, D. Knights, C.L. Lauber and J. Stombaugh, 2011. Microbial Biogeography of public restroom surfaces. PLoS ONE (h):e28/32.doi 10.1371//Journal.
11. Ryan, K.J. and C.G. Ray, 2004. Sherris Medical Microbiology (4th ed.). McGraw Hill, pp: 294-295.
12. Fisher, K and C. Philips, 2009. The Ecology, Epidemiology and Virulence of *Enterococcus*. Microbiology, 155(6): 1749-57.
13. Schleifer, K.H. and B.R. Kilpper, 1984. Transfer of Streptococcus faecalis and *Streptococcus faecium* to the genus *Enterococcus*. Int. J. Syst Bacteriol., 34: 31-34.
14. Scott, E.A. and S.F. Bloomfield, 1990a. The survival and transfer of microbial contamination via cloths, hands and utensils. Journal of Applied Bacteriology 68: 271-278.
15. Scott, E.A. and S.F. Bloomfield, 1990b. Investigation of the effectiveness of detergent washing, drying and chemical disinfection on contamination of cleaning cloths. Journal of Applied Bacteriology, 68: 279-283.
16. Scott, E.A., 1990. The survival and transfer of potentially pathogenic bacteria from environmental sites and surfaces, Ph. D thesis, University of London.
17. Guthrie, R.K., 1992. Salmonella. Florida, Boca Paton: CRC Press Inc., pp: 89.