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STUDIES ON THE SUSCEPTIBILITY OF COMMON UROPATHOGENS TO TOILET SEAT SANITIZERS AND THEIR ANTIBIOGRAM

ROY S[1*], DATTA A[2], GHOSH A[2], MANDAL G[2], BANERJEE S[2] AND ROY L[3]

1: Assistant Professor, Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata-700 016, West Bengal, India

2: 4th Year M.Sc. (5 Year Integrated) students, Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata-700 016, West Bengal, India

3: State-Aided College Teacher, Department of Microbiology, Sarsuna College (under University of Calcutta), 4/HB/A, Ho-Ch-Minh Sarani, Sarsuna Upanagari, Kolkata-700 061, West Bengal, India

***Corresponding Author: Souvik Roy: E-Mail: souvikroybiotech@sxccal.edu; Tel.: +91 98313 16389**

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ABSTRACT

Background: Unhygienic public washrooms are potential reservoirs of urinary tract infection (UTI)-causing pathogens. Recently, there is a surge of non-medical grade toilet seat sanitizers that claim to protect against UTIs. This work, a first of its kind, was done keeping in mind public health interests, as UTI is very common among women and children. **Objective:** To study the susceptibility of common uropathogens to toilet seat sanitizers, and determination of their antibiogram. **Materials and methods:** Toilet seat washes from ladies' public washrooms of a major railway terminal station, a tertiary care hospital, and a shopping mall were collected before and after spraying sanitizers. Each wash was cultured on nutrient agar for total heterotrophic count (THC), followed by cultivation on selective media to confirm the presence of the common uropathogens by standard microbiological and biochemical procedures. A comparative contamination analysis was made, and respective efficacies of sanitizers determined. The susceptibilities of the uropathogen isolates to some common and some lesser-used antibiotics was also studied. **Results and Discussion:** There was a significant reduction in THC on applying the toilet seat sanitizers, from which the relative efficacies of the sprays tested was calculated and compared. Several notorious uropathogens of public health concern were isolated from the toilet seat washes, among which *E. coli* showed multi-drug resistance towards three common antibiotics.

Conclusion: This makes this novel study clinically significant, showing the prevalence of antibiotic resistance in natural uropathogen isolates, which should strictly be avoided in treating UTIs that occur despite using sanitizers as preventive measures.

Keywords: Antibiogram, Toilet Seat Sanitizers, Uropathogens, Urinary Tract Infection

INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent bacterial infections, with about 150 million reported cases worldwide, and an incurred economic loss of about 6 billion US dollars **[1]**. UTIs are more prevalent in women, mainly because of the anatomy of their urinary tract **[2]**. The prevalence of UTI in pregnant women worldwide is reported to range from 3-24% **[3, 4]**. In a study conducted in Meerut, India, it was seen that the highest prevalence of UTIs in women was seen in the age group of 26-36 years (90.69%); however, in males, the highest susceptible age group to UTIs was ≥ 48 years (71.15%) **[5]**. This infection is also very common in children, with 3-8% of the cases occurring in girls, and 1% in boys, with an even greater risk for infants below the age of 6 months **[6]**.

In the etiology of the UTIs, the most prominent pathogen worldwide is *Escherichia coli* **[7]**. While there have been many newly emerging uropathogens, the main UTI-causing ones are members of the family Enterobacteriaceae (*Klebsiella*, *Enterobacter*, *Proteus*), and species of the genera *Pseudomonas*, *Vibrio*, *Staphylococcus*, *Enterococcus* and *Streptococcus* **[8]**.

Although there is a notion that bacteria are less likely to be found on cold hard surfaces such as toilet seats, research says otherwise **[9]**. Microbial aerosols created from flushing, improper cleaning of toilet seats after each use, and use of toilet by persons suffering from gastrointestinal diseases are some of the possible routes of addition of microbes to toilet seats **[10]**. A study to assess microbial flora present in public washrooms showed occurrence of a majority of bacteria such as *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio* spp., *Streptococcus faecalis*, and *Acinetobacter* spp. **[10]**. While many of these bacteria are uropathogenic, they have lesser chances of actually causing infections if proper washroom hygiene is maintained and toilet seats are cleaned regularly between each use **[11]**. However, in developing countries, such stringent practices cannot always be maintained due to socio-economic constraints, making the hygienic condition of public washrooms truly deplorable. This can lead to UTIs in users, especially in those with wound or compromised immunity **[12]**.

Toilet seat sanitizers are a relatively new concept with regard to sanitation and public hygiene, which are being widely advertised, marketed and promoted nowadays. These sanitize and deodorize when sprayed instantly from a certain distance, and claim to kill 99.9% uropathogens **[13]**. However, despite these huge claims, till date, no scientific studies have been conducted in any part of the world on the microbicidal efficacies of these sprays. Besides, increasing trends in antibiotic resistance among the common uropathogens is now a matter of great concern. A multi-centre study in Delhi, India, demonstrated extremely low susceptibility of the common uropathogens to some major first-line antibiotics like amoxicillin, amoxicillin / clavulanate, ciprofloxacin and co-trimoxazole **[14]**. According to a study conducted in Tamil Nadu, India, the occurrence of Extended spectrum beta-lactamase (ESBL) - producers in the urinary isolates of *E.coli* and *Klebsiella pneumoniae* was found to be 51.04% and 61% respectively **[15, 16]**. Hence, beta-lactam antibiotics were found to be ineffective against all of them **[15, 16]**. In another study performed in Aligarh, India, the common uropathogenic isolates were found to be resistant to ampicillin, chloramphenicol, erythromycin, rifampicin, sulphamathizole and tetracycline **[17]**.

Hence, taking into consideration the above scenario, our present work is the first and unique of its kind to study the comparative microbiological profile of the toilet seats of ladies' public washrooms from three distinct sites in the city of Kolkata (a major railway terminal station, a tertiary care hospital and a shopping mall) before and after sprays with five different toilet seat sanitizers, to identify and confirm the presence of some common UTI-causing pathogens (*E. coli* – a fecal coliform, *Pseudomonas* spp., *Vibrio* spp. and *Staphylococcus* spp.) from the samples collected therefrom, and as a result, getting an idea of the relative antimicrobial efficacies of the toilet seat sanitizers against them. Also, to get a notion of the existence, nature and degree of the spread of multidrug-resistance, antibiotic-susceptibility test of the uropathogen isolates was performed using some common, and some lesser-used antibiotics, and the respective antibiograms compared.

MATERIALS AND METHODS

Chemicals, reagents, dehydrated media, media base, supplements, antibiotics and toilet seat sanitizers

All chemicals and reagents used in this study were purchased from Merck Specialities

Private Limited, India and Sisco Research Laboratories Private Limited, India. All dehydrated media, media base, supplements and the antibiotics (amoxicillin, doxycycline, azithromycin, vancomycin and ciprofloxacin) in the form of commercially-available impregnated discs **(Table 1)** were procured from HiMedia Laboratories Pvt. Ltd., India.

Five popular toilet seat sanitizers (II-VI) to be used in this investigative study, two of which (III and IV) are manufactured by Multinational Companies (MNC) and the other three (II, V and VI) by India-based companies, and of varying chemical compositions, were purchased from commercial outlets in the city of Kolkata, India **(Table 2)**. Cotton pads, 5.5cm in diameter, were purchased from Dr. Morepen-Gubb (USA), New Delhi, India.

Sample collection

The study was conducted during summer, for over a period of two months (May-June 2019) in Kolkata, India. Toilet seat wash samples were collected from ladies' public washrooms from three different sites (sites 1-3) from three different geographical locations of the city, which are heavily thronged by common people all throughout the day - a major railway terminal station (site 1), a tertiary care hospital (site 2) and a shopping mall (site 3) **(Table 3)**.

Table 1: Antibiotic discs and their concentration (µg/disc)

ANTIBIOTIC DISC USED	CONCENTRATION (µg/disc)
Amoxicillin	30
Doxycycline	30
Azithromycin	15
Vancomycin	30
Ciprofloxacin	5

Table 2: Toilet seat sanitizers, their chemical compositions and unit price (₹/ml)

TOILET SEAT SANITIZER	CHEMICAL CONSTITUENTS	PRICE (₹/ml)	ORIGIN
II	*Eucalyptus globulus* oil, Citronella oil, Water and Excipients	1.98	India-based Company
III	Isopropyl Alcohol, Benzalkonium Chloride, Fragrance, Solvents & Propellant	2.00	Multinational Company
IV	Isopropyl Alcohol, Fragrance, Benzalkonium Chloride	2.40	Multinational Company
V	Isopropyl Alcohol, Propellant, Fragrance, Tea-tree oil	2.75	India-based Company
VI	Benzalkonium Chloride BP, Isopropyl Alcohol BP, Fragrance, Solvents & Propellant	2.00	India-based Company

Table 3: Sites of sample collection

SITE NO.	SITE	LOCATION IN KOLKATA CITY	TIME OF COLLECTION
1	Major railway terminal station	North Kolkata	10:00 A.M. - 11:00 A.M.
2	Tertiary care hospital	Central Kolkata	5:00 P.M - 6:00 P.M.
3	Shopping mall	South Kolkata	4:00 P.M. - 5:00 P.M.

Since UTIs are more prevalent in women and children, checking of the sanitation status of the toilet seats in the cubicles of the ladies' public washrooms, before and after sanitizer sprays, was the aim, as these are used not only by the ladies, but also much by the children accompanying them. From each of these three sites, six toilet seat wash samples were collected (samples I-VI), one control sample (I) collected before the application of the toilet seat sanitizer, and five experimental samples (II-VI) collected after the application of each of five different toilet seat sanitizers. Samples were collected between 10:00 A.M. and 11:00 A.M. from the railway station, between 5:00 P.M and 6:00 P.M. from the hospital, and between 4:00 P.M. and 5:00 P.M. from the shopping mall, which are their respective busiest hours. Each sample was collected under similar experimental conditions, as far as practicable. For collection of samples, 15ml of sterile 0.9% saline was taken in each sterile falcon tube. One sterile, round cotton pad was put in the saline solution in each of those falcon tubes, and the respective tubes taken to the sample collection sites. For all the three sites (sites 1-3), for each control sample (I), the cotton pad soaked in 0.9% saline was taken out from inside the respective falcon tube with a pair of sterile forceps, and slowly wiped over the surface of the toilet seat. The cotton pad was then put back inside the 0.9% saline in that particular falcon tube. For each of the five experimental samples (II-VI) to be collected from each site, each sanitizer was sprayed over the toilet seat from a 15cm distance and allowed to act for 10 seconds (as per manufacturers' instructions on the sanitizer spray bottles), and the sample collected in the same way as for the control one. During sample collection, hands were covered with sterilized gloves so as to avoid any kind of contamination. After collection, all the sample-containing falcon tubes were placed in clean, dry, sterilized plastic containers, which were tightly sealed and transported to the laboratory for analysis. Prior to analysis, all the samples were refrigerated (4^0C).

Sample preparation

During analysis, each cotton pad was taken out of the respective falcon tube, and squeezed against the wall of another empty, sterile falcon tube, till all its contents came out. Each cotton pad yielded 2ml of the toilet seat wash sample. When each cotton pad was taken out of the first falcon tube, the residual 0.9% saline left behind was 12ml. 2ml of each wash sample was then re-transferred to that 12ml of 0.9% saline in the first falcon tube, and mixed well. Each of these solutions is henceforth referred to as the sample-saline (1:7) mixture.

Total heterotrophic count (THC)

1ml of each of the sample-saline mixtures, for each site and each sanitizer spray, was then used for the determination of total heterotrophic count (THC) in terms of log_{10}CFU(colony forming units)/ml by standard Pour plate method, using nutrient agar (NA), and the plate incubated aerobically for 24 hours at 37°C **[18]**. Post-incubation, the number of CFUs and colony morphologies in each plate were noted.

Detection of uropathogens

For the detection of the presence of the most important UTI-causing bacteria, *E. coli*, in each of the sample-saline mixtures, 3×3 Most Probable Number or MPN test was carried out using double strength (2X) and single strength (1X) lactose broth (LB), and the tubes incubated in a rotary shaker for 48 hours at 37°C **[18, 19]**. MPN index (number of coliform bacteria/100ml) was then determined through observation of bacterial growth and gas bubble formation in the tubes **[20, 21]**. For detection of other selected uropathogens like *Pseudomonas* spp. and *Vibrio* spp., 0.1ml of each sample-saline mixture was inoculated into 5ml of Cetrimide broth (CB, primary enrichment medium for growth of *Pseudomonas* spp.) and 5ml of Peptone water (PW, primary enrichment medium for growth of *Vibrio* spp.) **[20]**. All the test tubes were thereafter incubated in a rotary shaker for 48 hours at 37°C, and observed for growth **[20]**.

Isolation of uropathogens

To isolate the selected UTI-causing pathogenic bacteria (*E. coli*, *Pseudomonas* spp. and *Vibrio* spp.), a loopful of inoculum from each of the 2X LB, CB and PW tubes showing visible growth was streaked onto the selective media plates like Eosin Methylene Blue Agar (EMB), *Pseudomonas* Agar Base (PAB, with glycerol, C-F-C supplement and CetriNix supplement) and Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS) respectively **[9, 20]**. For the isolation of the uropathogenic *Staphylococcus* spp., a loopful of each sample-saline mixture was streaked onto the selective Baird Parker Agar (BP, with egg yolk tellurite emulsion supplement) plate **[9, 20]**. All the plates were thereafter incubated aerobically for 24 hours at 37°C, following which the growth of the uropathogens were noted, including the colony types and their morphological characteristics **[9, 20]**. The typical colonies of the uropathogens isolated were subsequently sub-cultured twice on their respective selective media plates to purify and obtain pure cultures in the form of single, isolated colonies. The single colonies so obtained, with distinct characteristic features of the uropathogens (named as P1VId, T1Vb, T1Vc, $B^1$1IIb, $B^1$1Vc, E1Ic, $E^1$1IIc, $E^2$1IIa, $E^2$1Vf and $E^2$1VIg) were grown in NB tubes, and all the pure cultures refrigerated (4^0C) for further use.

Confirmation of uropathogens

The isolated pure culture of each of the selected uropathogens (*E. coli*, *Pseudomonas* spp., *Vibrio* spp. and *Staphylococcus* spp.) were confirmed by standard microbiological procedures including Gram-staining, and by a battery of characteristic biochemical tests including starch hydrolysis (SH), urea hydrolysis (UH) and gelatin hydrolysis (GH), nitrate reduction (NR), catalase production (CP), oxidase production (OP), C-source (glucose, sucrose and fructose) utilization (growth in Triple Sugar Iron Agar, TSI), gas and H_2S production (in TSI agar), and response in Indole (I), Methyl Red (MR), Voges-Proskauer (VP) and Citrate Utilization tests (CUT) (IMViC) **[21, 22]**. Due to scarcity of time and infrastructural constraints, confirmation by ribotyping and serotyping could not be completed during the tenure of the study.

Antibiotic susceptibility test

Susceptibility test of the ten uropathogen isolates towards five antibiotics, some common, and some lesser-used, was performed according to the standard Kirby–Bauer Disc Diffusion Assay method, as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI) **[23, 24]**. The commercially-available, different antibiotic-impregnated paper discs used in the study included amoxicillin (AMX, 30µg/disc), doxycycline (DO, 30µg/disc), azithromycin (AZM, 15µg/disc), vancomycin (VA, 30µg/disc) and ciprofloxacin (CIP, 5µg/disc).

Statistical analysis

All results were expressed as mean ± SEM (Standard Error of Mean) for an individual experiment. Each experiment was performed three times (n=3), and the mean value from all the sets of those experiments presented **[25]**. Student's t-test was performed as applicable in each case, and the values were found to be significant at 5% probability level (P 0.05) **[26]**.

Computation, data analysis and graphics

Windows Microsoft Word 2010 and Windows Microsoft Excel 2010 software were employed for all computation, data analysis and graphics.

RESULTS

Total heterotrophic count (THC)

Colony morphologies **(Figure 1)** and the respective THC (log_{10}CFU/ml) **(Table 4)** were noted for each of the NA plates for each of the samples.

The respective percent reductions **(Figure 2a, Table 4)** were calculated from the corresponding THC values, and their mean values considered for representing the percent efficiency of each of the sanitizers (II-VI) in reducing microbial growth **(Figure 2b, Table 4) [27]**.

Detection, isolation and confirmation of uropathogens

A similar, but not identical, trend in data was also seen for the MPN index (number of coliform bacteria/100ml). From the LB tube observations **(Figure 3)**, a respective MPN index was assigned to each of the samples, according to the standard 3×3 MPN chart **(Table 5)**.

The growth of the uropathogens from each of the control (I) and experimental samples (II-VI) from each site (1-3) were noted on the respective selective media plates (EMB, PAB, TCBS and BP), including their typical colony morphologies **(Figure 4, Table 6)**.

The uropathogens were then confirmed by standard microbiological procedures including Gram staining **(Table 6)**, and by some characteristic biochemical tests **(Figure 5, Table 7)**.

Antibiotic susceptibility test

From the Kirby–Bauer Disc Diffusion Assay with the five antibiotics **(Figure 6)**, the average diameter (in mm) of the respective zones of growth inhibitionin case of each of the ten uropathogen isolates was calculated **(Figure 7)**.

Based on the average diameter of their respective zones of inhibition for each of the five different antibiotics, using the Standard Kirby-Bauer Zone Size Data Interpretative Chart, the ten uropathogen isolates were classified as either resistant (R), intermediately susceptible (I) or completely susceptible (S) to each of the five antibiotics used **(Figure 8) [28]**.

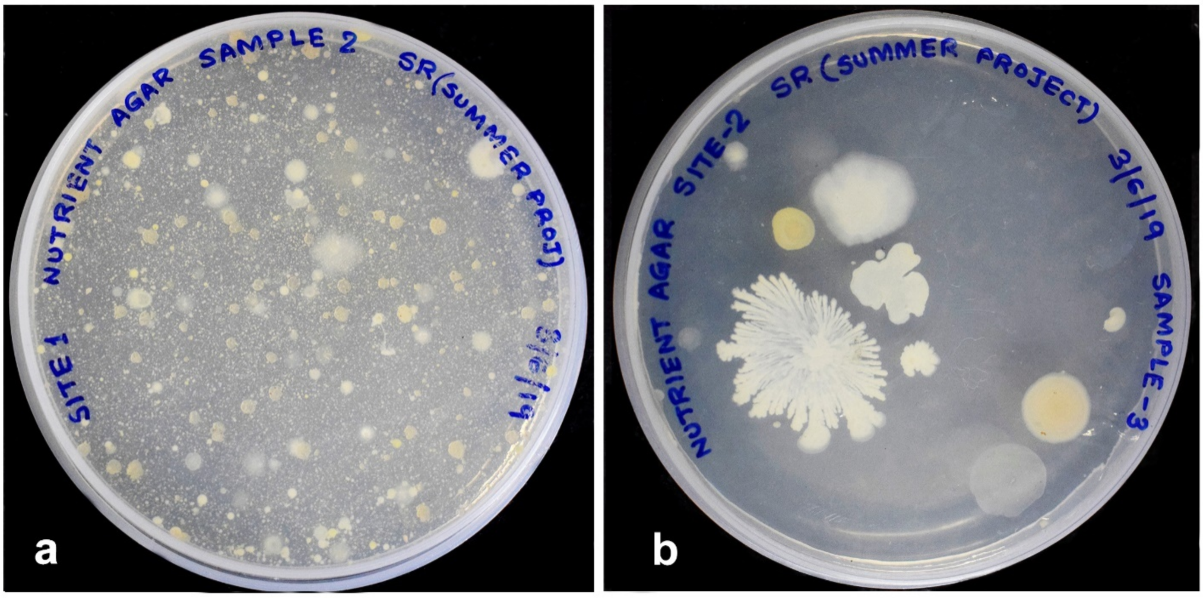

Figure 1: NA Plates showing the growth of various microorganisms from the plated samples: a: Site 1, Sample II; b: Site 2, Sample III

Table 4: Total Heterotrophic Count (THC, log_{10}CFU/ml), log_{10} reduction in CFU/ml, percent reductions and mean percent efficiencies of the toilet seat sanitizers

TOILET SEAT SANITIZER	SITE NO.	THC (log_{10}CFU/ml)		log_{10} REDUCTION IN CFU/ml	PERCENT REDUCTION (%)	MEAN PERCENT EFFICIENCY (%)
		BEFORE SANITIZER SPRAYING	AFTER SANITIZER SPAYING			
II	1	3.81	3.51	0.30	49.88	83.16
	2	3.77	0.60	3.17	99.93	
	3	2.92	0.30	2.62	99.76	
III	1	3.81	1.71	2.10	99.21	99.52
	2	3.77	1.23	2.54	99.71	
	3	2.92	0.48	2.44	99.64	
IV	1	3.81	2.47	1.34	95.43	98.29
	2	3.77	1.04	2.73	99.81	
	3	2.92	0.48	2.44	99.64	
V	1	3.81	3.47	0.34	54.29	84.02
	2	3.77	1.60	2.17	99.32	
	3	2.92	1.04	1.88	98.68	
VI	1	3.81	2.47	1.34	95.43	97.88
	2	3.77	0.77	3.0	99.90	
	3	2.92	1.15	1.77	98.30	

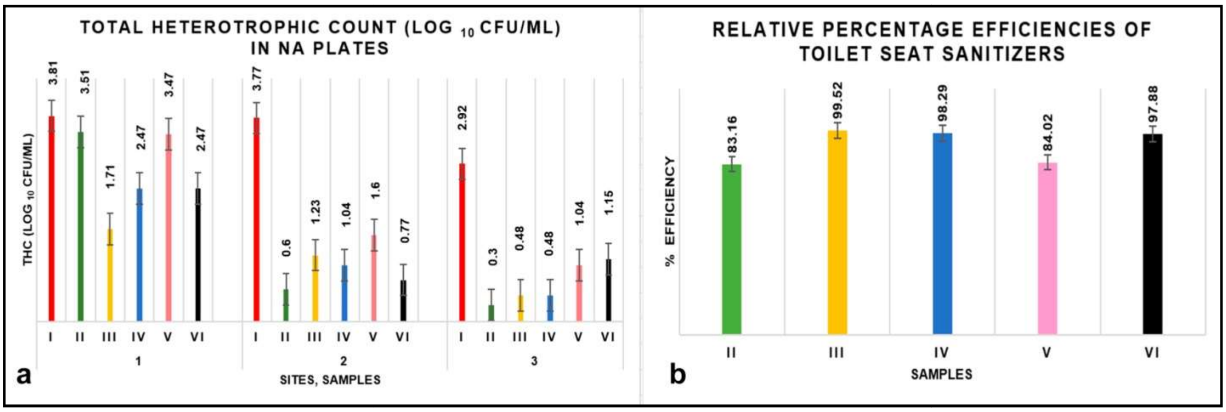

Figure 2: a: Graphical representations of relative THC (log_{10}CFU/ml) in the Control (I) and Experimental samples (II-VI) from 3 (1-3) sites of collection; b: Relative percent efficiencies of different toilet seat sanitizers (II-VI)

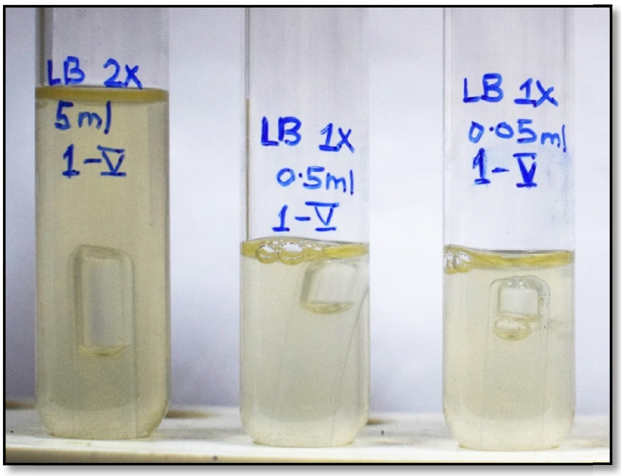

Figure 3: Representative result of 3×3 MPN test

Table 5: MPN Index (number of coliform bacteria/100ml) of Control (I) and Experimental samples (II-VI)

SITE NO.	SAMPLE	MPN INDEX (NUMBER OF COLIFORM BACTERIA/100ml)
1	I	>2400
	II	>2400
	III	23
	IV	<1
	V	>2400
	VI	>2400
2	I	>2400
	II	240
	III	<1
	IV	3
	V	23
	VI	23
3	I	240
	II	23
	III	240
	IV	<1
	V	23
	VI	<1

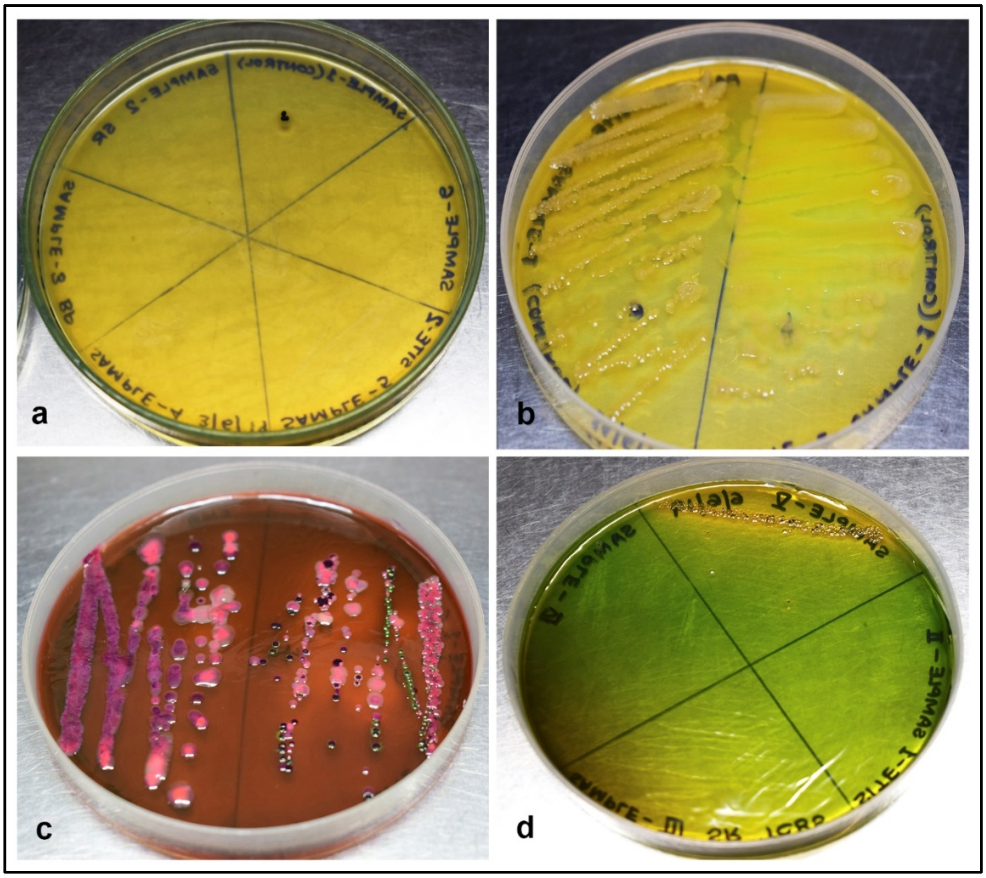

Figure 4: Representative selective media plates for different pathogens: a: BP plate showing growth of *Staphylococcus* spp. b: PAB plate showing growth of *Pseudomonas* spp. c: EMB plate showing growth of coliforms and non-coliforms d: TCBS plate showing growth of *Vibrio* spp.

Table 6: Characterization of the Uropathogens by Colony characteristics and Gram staining observations

UROPATHOGEN	COLONY CHARACTERISTICS	GRAM STAINING OBSERVATIONS (400X)
P1VId	Translucent colonies producing green pigment	Medium sized rod-shaped cells in single arrangement
T1Vb	Yellow colonies, with dark black nucleation	Small comma shaped cells in single arrangement
T1Vc	Yellow colonies	Small comma shaped cells in single arrangement
$B^1$1IIb	Black colonies, slightly appressed	Small coccus shaped cells in cluster arrangement
$B^1$1Vc	Grey colonies, raised in appearance	Small coccus shaped cells in cluster arrangement
E1Ic	Translucent colonies producing a green sheen	Medium sized rod-shaped cells in single arrangement
$E^1$1IIc	Purple colonies, mucoid in nature	Medium sized rod-shaped cells in single arrangement
$E^2$1IIa	Pink colonies, mucoid in nature	Medium sized rod-shaped cells in single arrangement
$E^2$1Vf	Colorless colonies	Medium sized rod-shaped cells in single arrangement
$E^2$1VIg	Colorless colonies	Medium sized rod-shaped cells in single arrangement

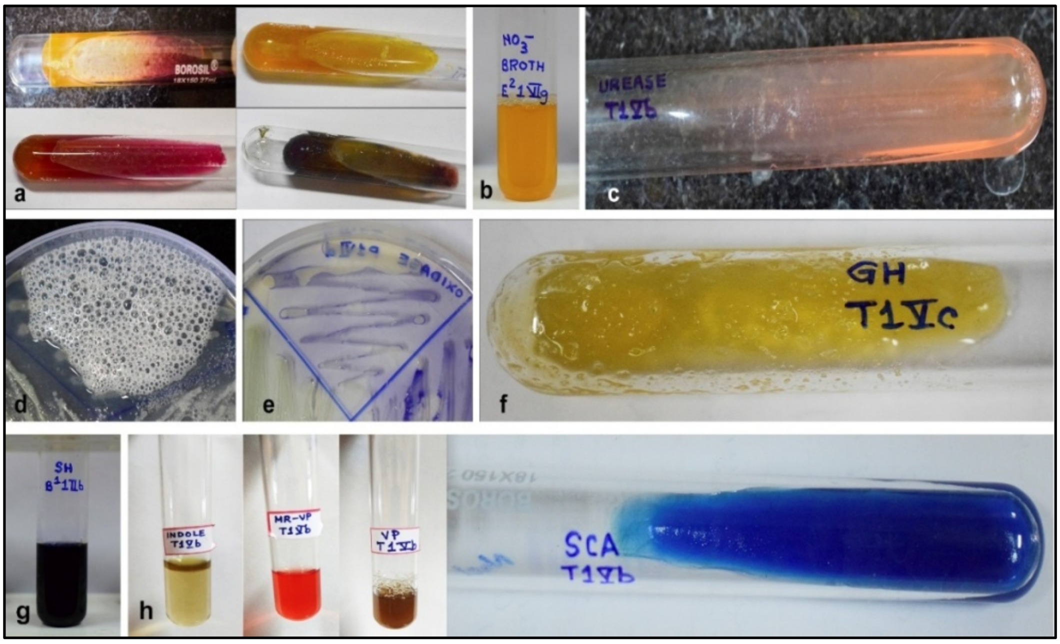

Figure 5: Representative biochemical test results of the ten isolated uropathogens in: a: TSI; b: NR; c: UH; d: CP; e: OP; f: GH; g: SH; h: IMViC

Table 7: Genus identification by Biochemical tests

URO-PATHOGEN	TSI SLANT			NR	UH	CP	OP	GH	SH	IMVIC				GENUS IDENTIFIED
	SLANT/BUTT*	H_2S	GAS							I	MR	VP	CUT	
P1VId	K/K	Nil	Nil	+	Nil	+	+	+	Nil	Nil	Nil	Nil	+	*Pseudomonas*
T1Vb	K/A	+	+	+	+	+	Nil	+	Nil	Nil	+	Nil	+	*Proteus*
T1Vc	K/A	Nil	Nil	+	Nil	+	+	+	+	+	Nil	Nil	Nil	*Vibrio*
B[1]1IIb	A/A	Nil	Nil	+	+	+	Nil	+	Nil	Nil	Nil	+	+	*Staphylococcus*
B[1]1Vc	A/A	Nil	Nil	+	+	+	Nil	+	Nil	Nil	+	+	Nil	*Staphylococcus*
E1Ic	A/A	Nil	+	+	Nil	+	Nil	Nil	Nil	+	+	Nil	Nil	*Escherichia*
E[1]1IIc	A/A	Nil	+	+	Nil	+	Nil	Nil	Nil	Nil	Nil	+	+	*Enterobacter*
E[2]1IIa	A/A	Nil	+	+	Nil	+	Nil	Nil	Nil	Nil	Nil	+	+	*Enterobacter*
E[2]1Vf	K/A	Nil	+	+	Nil	+	Nil	Nil	Nil	Nil	+	Nil	+	*Salmonella*
E[2]1VIg	K/A	Nil	+	+	Nil	+	Nil	Nil	Nil	Nil	+	Nil	+	*Salmonella*

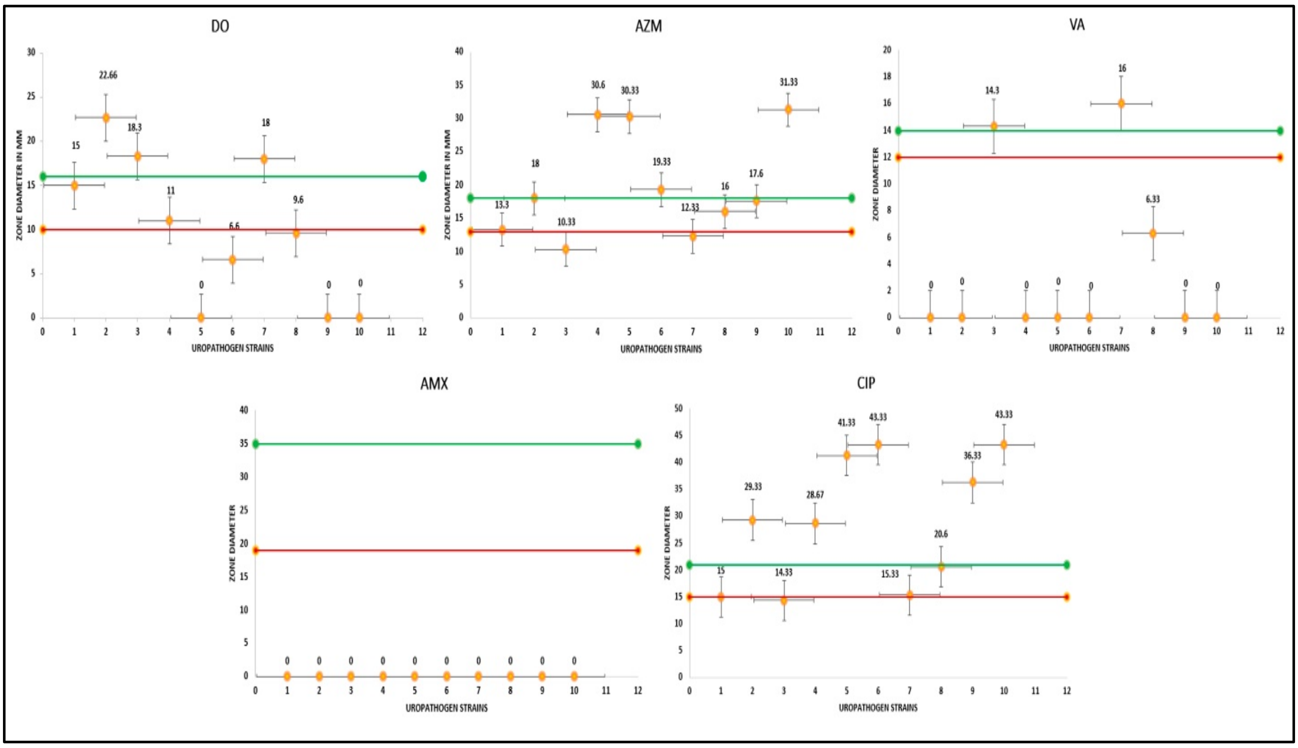

Figure 6: Scatter plot showing the diameters of the zones of growth inhibition (mm) and the cut-off lines for susceptibility (above the green line), intermediate susceptibility (between the green and red lines) and resistance (below the red line) of the ten isolated uropathogens towards the five antibiotics used; along X axis: 1 - E1Ic; 2 - $E^2$1VIg; 3 - $B^1$1IIb; 4 - $E^1$1IIc; 5 - T1Vb; 6 - P1VId; 7 - $B^1$1Vc; 8 - T1Vc; 9 - $E^2$1Vf; 10 -$E^2$1IIa

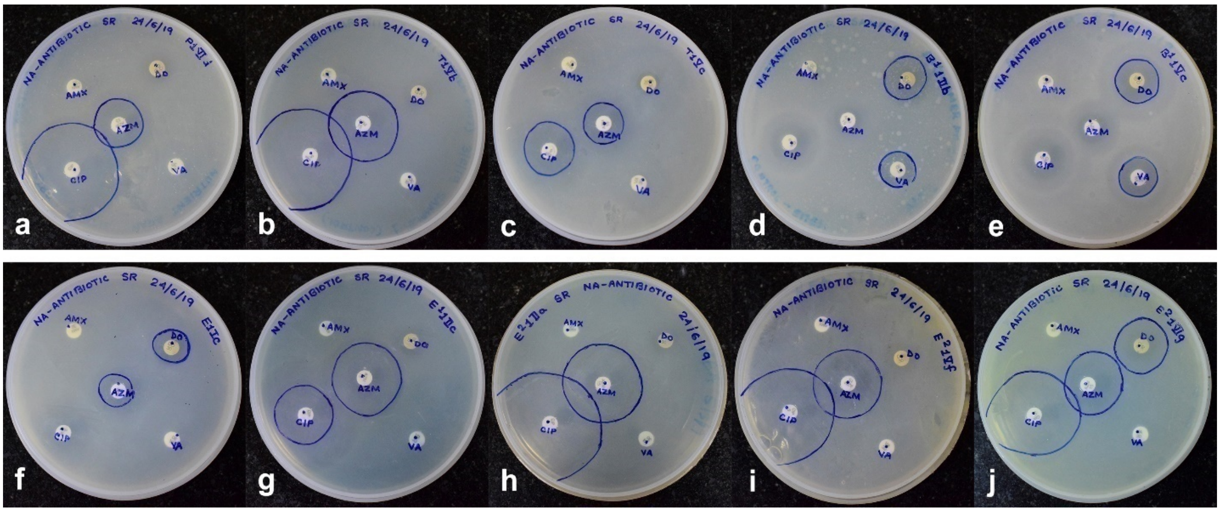

Figure 7: NA plates showing corresponding zones of inhibition of growth of the ten isolated uropathogens by five antibiotic discs: a: P1VId; b: T1Vb; c: T1Vc; d: $B^1$1IIb; e: $B^1$1Vc; f : E1Ic; g: $E^1$1IIc; h: $E^2$1IIa; i: $E^2$1Vf; j: $E^2$1VIg

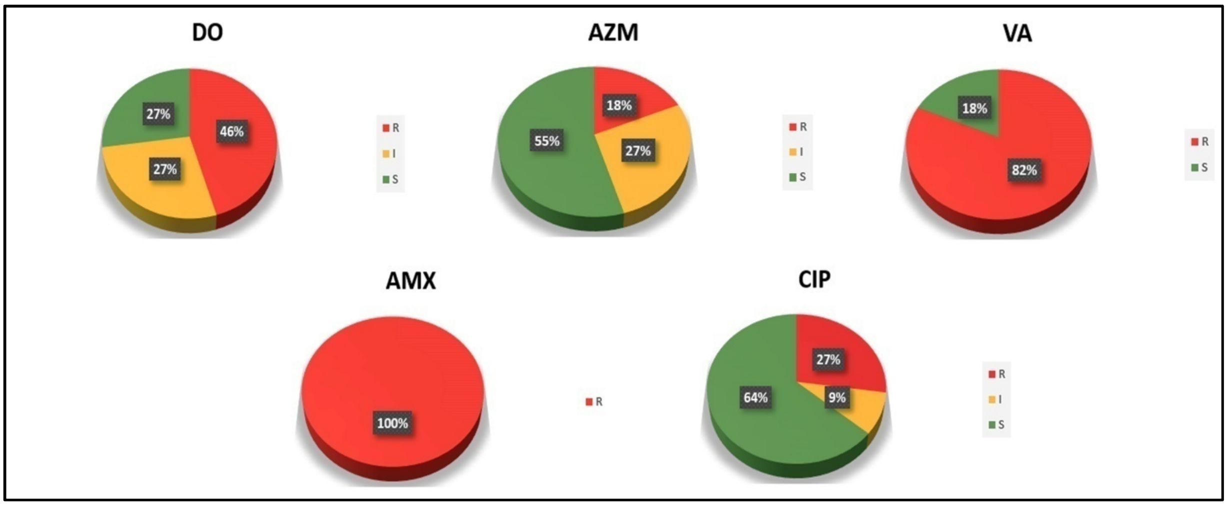

Figure 8: Pie charts showing the percentage (%) of completely susceptible (S), intermediately susceptible (I) and resistant (R) uropathogen isolates for the five antibiotics used

DISCUSSION

The control sample (I) collected from site 1 (ladies' public washroom in the major railway terminal station) recorded the highest THC (log_{10}CFU/ml) of 3.81 and a high MPN index (>2400), control sample (I) collected from site 2 (ladies' public washroom in the tertiary care hospital) showed an intermediate THC (log_{10}CFU/ml) of 3.77 and a high MPN index (>2400), whereas control sample (I) collected from site 3 (ladies' public washroom in the shopping mall) exhibited the least THC (log_{10}CFU/ml) of 2.92 and least MPN index (240) **(Table 4, Table 5)**. This is quite logical considering that the ladies' public washroom in the major railway terminal station is frequented by a huge number of women (and children), with cleaning in between even a few uses being neither feasible nor practicable. In case of the shopping mall however, the washrooms are generally cleaned regularly between a few successive uses. Ladies' public washroom in the tertiary care hospital showed an intermediate THC value, denoting an average possible maintenance of hygiene. Keeping in mind that the aforesaid THC values also included most of the notorious uropathogens isolated in huge numbers, the above observations suggest that if proper hygiene is maintained within the public washrooms, and they are regularly washed, cleaned and disinfected at regular intervals, there will be much lesser chances of spread of UTIs among the users.

Reduced THCs (in all the 3 sites) **(Table 4)** and reduced MPN indices (particularly in site 2 and site 3) **(Table 5)** in case of the experimental samples II-VI, with respect to their respective controls (I), showed that the antimicrobial formulation of the toilet seat sanitizers helped in killing at least some of the uropathogens. The sanitizers were potent in reducing the growth of the uropathogens, but with varying efficiencies (83.16% - 99.52%) **(Figure 2, Table 4)**. However, none of them could kill 99.9% of the microorganisms, as claimed. The efficiency of sanitizer III in reducing microbial growth on the toilet seats was found to be closest to this claim, with the highest mean efficiency of 99.52%, while sanitizer II turned out to be least potent with the least mean efficiency of 83.16% **(Figure 2, Table 4)**. Based on their principal chemical constituents, it seems that sanitizers II and V, consisting of natural essential oils (*Eucalyptus globulus* oil and Tea-tree oil respectively) as the main ingredient, had the poorest performances with mean efficiencies of 83.16% and 84.02% respectively, whereas sanitizers III, IV and VI containing principal chemical components like benzalkonium chloride and isopropyl alcohol (which are widely used in disinfectants and sanitizers), showed more or less comparable efficiencies to each other (mean efficiencies of 99.52%, 98.29% and 97.88% respectively), while exhibiting relatively better performances than sanitizers II and V **(Figure 2, Table 1, Table 4)**. Also, no linear correlation was found to exist between the price and effectiveness of the sanitizers. Sanitizer II, priced at 1.98 ₹/ml (least expensive) and sanitizer V, priced at 2.75 ₹/ml (most expensive), both performed poorly **(Figure 2, Table 1, Table 4)**.

Hence, this novel study, for the very first time, shows that the toilet seat sanitizers are a good measure of chemical control against the common uropathogenic bacteria, especially for the women commuters while travelling, as in our study conducted, all of them gave more-or-less satisfactory performances (83.16% - 99.52%) in killing most of the UTI-causing notorious pathogens. Sanitizers can thus be safely used to restrict the spread of UTIs contracted from soiled toilet seats of public washrooms. In future, further work that needs to be done is to go for altering and re-formulating the chemical composition of the toilet seat sanitizers, of those which are used in our study and some newer ones as well, and then determine their effectiveness against the same set or may be a new set of UTI-causing pathogens, so that the health care sector as well as the common public, particularly women, become aware of the use and potential efficacy of these sanitizer sprays in limiting the outbreak of this fatal disease in them.

CONCLUSION: UTIs often manifest fatal as the drug-resistant (DR) variety, and this complication is attributed to the involvement of multidrug-resistant (MDR) uropathogen strains as found in the form of natural isolates in this study. MDR bacteria are notorious, due to the major difficulties involved in excavating and applying the most suitable treatment regime for curing the fatal diseases they are associated with. The number and variety of antibiotic-resistant uropathogenic bacteria reported in this study is highly alarming, many of them being resistant to multiple antibiotics i.e. MDR **[29, 30] (Figure 7)**. All the natural uropathogenic isolates obtained in this study were found to be resistant to AMX **(Figure 7, Figure 8)**, which might be a consequence of doctors prescribing it for almost any common infection, including UTIs **[31]**. Resistances of the uropathogens to CIP and AZM were found to be comparatively lesser, excepting *Escherichia coli* and *Staphylococcus* spp. **(Figure 7, Figure 8)**. Antibiograms for VA and DO showed that the corresponding uropathogen susceptibilities are intermediate, with every possibility of the drugs becoming ineffective very soon against the target microbes **(Figure 7, Figure 8)**. In future, a periodic antibiogram profiling in every part of the world is thus required to formulate and re-design the necessary therapy for DR-UTIs. This makes the present study immensely significant clinically as it includes an indication of the antibiotic resistance patterns prevalent in natural uropathogen isolates, at least in this part of India, which can be used as reference information for treating both drug-sensitive (DS) - and DR-UTIs that can occur despite adoption of all possible precautionary measures, including the use of toilet seat sanitizers.

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