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Biofilm formation and antibiotic resistance of urinary catheter associated bacteria from hospitalized patients, Bangladesh

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ABSTRACT

Aims: Biofilm formation of bacteria inside the surface of urinary catheters triggers severe urinary tract infections (UTIs). This study aims to determine the biofilm forming capacity of bacteria isolated from urinary catheters of patients diagnosed with UTIs as well as comparison of antibiotic sensitivity patterns between biofilm and non-biofilm forming isolates.
Methodology and results: A total of 40 urinary catheters were collected from 96 h catheterized patients. The isolated uropathogenic bacteria were identified and examined for biofilm formation using the microtiter plate method. Later, the isolates were subjected to antimicrobial susceptibility towards 12 antibiotics that commonly used for treating UTIs using the disk diffusion method. All the catheters were found colonized with two to five different bacterial species individually. Out of the 131 isolates from 40 catheters, *Pseudomonas aeruginosa* (38/131, 29%) was the predominant isolated bacteria followed by *Escherichia coli* (31/131, 24%), *Proteus vulgaris* (24/131, 18%), *Klebsiella pneumoniae* (21/131, 16%) and *Staphylococcus aureus* (17/131, 13%). Among these, the highest biofilm forming capacity was observed in *P. aeruginosa* (26%), followed by *P. vulgaris* (16%) and *K. pneumoniae* (13%). Regarding antibiotic resistance, biofilm forming bacteria showed resistance to multiple drugs except for carbapenems. Moreover, biofilm formers exhibited higher resistance than non-biofilm formers against antibiotics such as trimethoprim/sulfamethoxazole (100% vs 82%), amoxicillin-clavulanic acid (81% vs 55%), cefixime (85% vs 55%), ceftriaxone (81% vs 45%), cefalexin (93% vs 55%), amikacin (70% vs 45%), ampicillin (89% vs 73%), ciprofloxacin (70% vs. 36%) and ceftriaxone (81 vs 45%), (p-value<0.05).
Conclusion, significance and impact of study: Most of the isolated uropathogenic bacteria from catheters were biofilm formers and multiple antibiotic resistant. Appropriate selection of antibiotics, meticulous hygiene practices in hospital settings and limiting the duration of catheterization can reduce biofilm formation and the emergence of antibiotic resistance.

Keywords: Urinary catheter, biofilm, antibiotic resistance, biofilm formers, non biofilm formers

INTRODUCTION

One of the leading causes of morbidity and mortality throughout the world is infections associated with hospital set up commonly known as hospital-associated infections (HAIs) which includes surgical site infections, bloodstream infections, urinary tract infections (UTIs), respiratory infections, gastroenteritis, pneumonia and meningitis and other soft tissue infections (Nicolle, 2012; Feleke *et al.*, 2018; Haque *et al.*, 2018). It was reported that about 40% of all HAIs are UTIs with a significant rate of morbidity and mortality (Haque *et al.*, 2018). This percentage is also prominent in developing countries like Ethiopia (68.71%) (Ali *et al.*, 2018), Nigeria (43%) (Ige *et al.*, 2011; Iliyasu *et al.*, 2018) as well as in Bangladesh (15.4%) (Afroz *et al.*, 2017; Alam *et al.*, 2019).

Several risk factors like age, pregnancy, diabetics, neurogenic bladder, menopause, frequent intercourse, change of the local bacterial flora, history of UTIs during pre-menopause or in childhood, family history have been made known to trigger UTIs (Storme *et al.*, 2019). On top of this, catheterization is also a leading source of UTIs and comprises around 80% of all hospital-associated UTIs (Jacobsen *et al.*, 2008; Sabir *et al.*, 2017). Catheters are standard medical devices that commonly used in hospitals to retain urine and eliminate urinary irregularities from patients. The predominant pathogens associated with catheter associated urinary

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tract infections (CAUTIs) include *Escherichia coli*, *Enterococci*, *Serratia* spp., *Providencia stuartii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*, *Enterobacter* sp., *Proteus mirabilis*, *Morganella morganii* and coagulase-negative Staphylococci (Nicolle, 2012; Sabir *et al.*, 2017; Maharjan *et al.*, 2018). These pathogens can become a threat to treatment failure because of the emergence of multidrug resistance. The heavy use of wide-spectrum antibiotics to any infections has puts a high selective pressure that triggers the emergence of multidrug resistant bacteria (Soltani *et al.*, 2016). This situation becomes jeopardizes when these resistant bacteria form biofilms and cause persistent infections (Almalki and Varghese, 2020). Biofilm, a layer of microorganism that composed of a complex assembly of bacterial protein, polysaccharide and DNA in an extracellular polymeric matrix and can be found on various surfaces, including, natural aquatic or potable water systems, living tissues and medical devices (O'Toole *et al.*, 2000; Awoke *et al.*, 2019). The surface of the urinary catheter also provides opportunities for microorganisms to form biofilms. Biofilm helps bacteria to survive, spread and resist antimicrobial therapy (Donlan, 2002). Several studies mentioned that biofilm-formers exhibited 10–1000 times more resistance to antimicrobials compare to non-formers (Potera, 2010; Sharma *et al.*, 2019).

This study aimed to assess the distribution of catheterized bacterial pathogens, their biofilm-forming capacity as well as the comparison of antibiotic sensitivity patterns between biofilm and non-biofilm-forming isolates.

MATERIALS AND METHODS

Collection of samples

A total of 40 urinary catheters were collected randomly from 96 h catheterized postoperative female patients diagnosed with urinary tract infections and preserved in sterile polybag at 4 °C for 6 h. Samples were collected from two hospitals of Chittagong, Chittagong Medical College Hospital (CMCH) and University of Science and Technology Hospital, Chittagong (USTC), Bangladesh.

Catheters were cut into pieces of 1 cm in length, rinsed with sterile phosphate-buffered saline (PBS, pH 7.4). Then, 1 mL of the PBS was transferred into 5 mL of Brain Heart Infusion broth (BHIB) (Himedia M210, India) and incubated at 37 °C for 24 h.

Isolation and identification of bacteria

One loopful suspension from BHIB for each catheter sample was streaked on different selective media like Cetrimide agar (Oxoid™ CM0579, UK), MacConkey agar (Oxoid™ CM0007, UK), Casein Enzyme Hydrolysate (CLED) agar (Oxoid™ CM0301, UK) and Mannitol Salt Agar (Himedia™ M118, India) for selective isolation of *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. aureus.* Then, the plates were incubated at 37 °C for 24 h. The individual isolates obtained from the selective media plates were presumptively identified using conventional microbiological methods including both culture-based methods and biochemical tests, respectively according to Bergey's Manual, 9th Edn. (Holt *et al.*, 1994).

Quantitative detection of biofilm formation

Stepanović *et al*. (2007) method was used for quantitative analysis of biofilm formation. To conduct the test, each bacterial isolate was inoculated into 10 mL of trypticase soy broth (Himedia LQ508) in addition with 1% glucose and incubated at 37 °C for 24 h. After that each culture was diluted for 100 times using fresh trypticase soy broth medium at room temperature. Each diluted culture was transferred to individual wells of sterile 96-well flat-bottom polystyrene tissue culture-treated plates (Sigma Aldrich, Costar, USA) in the amount of 125 µL and incubated at 37 °C for 24 h. The negative control wells contained same amount of fresh trypticase soy broth medium. After incubation, the contents of each well were gently tapped out. Each well was washed four times with 0.2 mL of phosphate buffer saline (PBS) (pH 7.2) to remove the free-floating bacteria from the wells before fixation. Finally, the wells were treated with sodium acetate (2%) for cell fixation. After fixation, the plate with sodium acetate was washed with 0.2 mL PBS thrice before staining. For staining, 125 µL of 0.1% crystal violet was added for five min. The excess crystal violate solution was removed and the plate was rinsed 3-4 times with PBS. For elution, 125 µL ethanol-acetone mixture (80:20) was used and left at room temperature for 30 min. The optical density (OD_{630}) of the stained adherent biofilm was determined by using a micro ELISA auto reader (Model 680, Biorad, UK) and analyzed for calculating the biofilm-forming capacity of the bacterial isolates. The experiment was performed three times in triplicates and the results were interpreted according to the criteria as described by Stepanović *et al.* (2007).

In brief, OD_{630} values were calculated as OD_{630} = Ø OD – ODc, where Ø OD indicates optical density of each well containing the bacterial isolate and ODc indicates the cut-off value which was calculated using the following formula: ODc = Ø ODn + 3 × SDn. Here, Ø ODn indicates the mean of negative control and SDn indicates the standard deviation of the negative control. The isolates were categorized based on the OD_{630} values:

$OD_{630} \leq$ ODc: No biofilm
ODc < $OD_{630} \leq$ 2× ODc: Weak biofilm
2× ODc ≤ $OD_{630} \leq$ 4× ODc: Moderate biofilm
4× ODc < OD_{630}: Strong biofilm

Antibiotic susceptibility patterns

Antibiotic susceptibility pattern of all isolated bacteria was screened using the disc diffusion method, towards Ampicillin, AMP (10 µg), Amoxicillin/Clavulinic acid, AMC (30 µg), Amikacin, AK (30 µg), Gentamicin, CN (30 µg), Erythromicin, E (15 µg), Cephalexin, CL (30 µg),

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Cefixime, CFM (5 µg), Ceftriaxone, CRO (30 µg), Ciprofloxacin, CIP (5 µg), Trimethoprim/sulfamethoxazole, SXT (1.25/23.75 µg), Imipenem, IPM (10 µg) and Meropenem, MEM (10 µg). The inhibition zone diameter against tested antibiotics was measured in mm and interpreted based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). *Pseudomonas* spp. ATCC 27853 was used as control strains in this study.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) software version 22 (SPSS Inc, Chicago, IL, USA). The chi-squared test, odds ratio (risk) with 95% CI, was used to calculate possible associated factors between the resistance of biofilm formers and non-biofilm formers. All statistical test values of $p<0.05$ were considered statistically significant.

RESULTS

Catheter colonization and distribution of uropathogens

All the urinary catheter samples collected were found to be culture-positive with two to five mixed bacterial species. The number of catheters with mixed colonization is summarized in Figure 1, which most of the catheters (n=18) show co-colonized with 3 species and the least number of catheters (n=3) shows co-colonized with 5 species. From 40 urinary catheters, a total of 131 bacterial isolates were isolated using selective media. Out of the 131 identified isolates, the most predominant bacteria were *P. aeruginosa* (38/131, 29%) followed by *E. coli* (31/131, 24%), *P. vulgaris* (24/131, 18%), *K. pneumoniae* (21/131, 16%) and *S. aureus* (17/131, 13%).

Assessment of biofilm formation ability of isolates

Next, the biofilm formation capability of these isolates was examined using the microtiter plate method. Out of 131 isolates, 71% (n=93) of them showed biofilm forming capacity. Among these, 87% (81/93) exhibited weak to moderate biofilm formation and 12.9% (12/93) exhibited strong biofilm formation (Table 1). If we linked the catheter colonization and biofilm forming ability, all the isolates found in catheter co-colonized with four to five different bacterial species were capable of forming biofilms. Among the 18 catheters that were co-colonized with three bacterial species, ten of them were colonized with biofilm forming bacteria. Among all the biofilm forming bacteria, *P. aeruginosa* (89.5%) was the most prevalent biofilm-former, followed by *P. vulgaris* (87.5%) and *K. pneumoniae* (85.7%) (Table 1). The optical density (OD_{630}) of all 93 biofilm formers is presented in Supplementary Table S1.

Antibiotic susceptibility test of biofilm forming isolates

In this study, 12 antibiotics were used to evaluate antibiotic sensitivity of catheter associated biofilm forming bacteria. All the biofilm-formers showed resistance to multiple antibiotics except for both carbapenems (meropenem and imipenem). The antibiotic pattern of each type of biofilm producer was recorded in Table 2. All *P. vulgaris* isolates showed pan resistance to 10 treated antibiotics. Other bacterial isolates such as *P. aeruginosa* have also shown pan resistance to ampicillin, cephalexin, cefixime and trimethoprim/sulfamethoxazole. Apart from these two, all *K pneumoniae* isolates were also resistant to ampicillin, amoxicillin/clavulanic acid, erythromycin and trimethoprim/sulfamethoxazole (Table 2). Gram-positive isolates *S. aureus* showed less resistance than all Gram-negative isolates. However, all the isolates of *S. aureus* showed pan resistance to cefixime and trimethoprim/sulfamethoxazole.

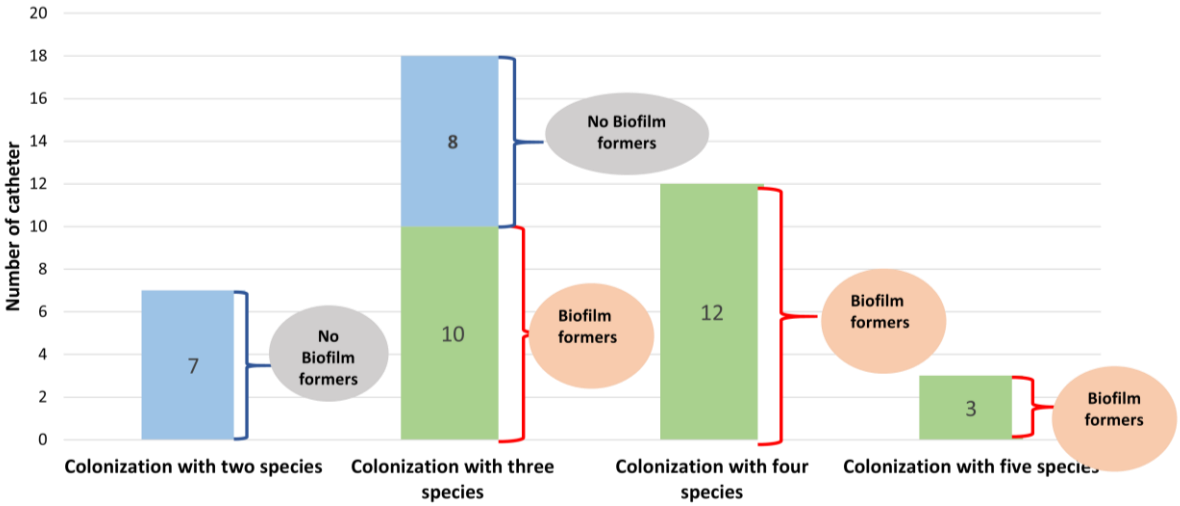

Figure 1: Number of catheters colonization by multiple species and biofilm formers.

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Table 1: Biofilm formation capacity of isolated to pathogen (n=131) using microtiter plate method.

Bacteria	Biofilm formation*							
	Formers (n=93) (71%)					Non-formers (n=38) (29%)		
	Strong	Moderate	Weak	n	%	n	%	Total
P. aeruginosa	6	9	19	34	89.5	4	10.5	38
E. coli	2	1	7	10	32.3	21	67.7	31
P. vulgaris	3	8	10	21	87.5	3	12.5	24
K. pneumoniae	1	4	13	18	85.7	3	14.3	21
S. aureus	0	2	8	10	58.8	7	41.2	17
Total (%)	12 (12.9%)	24 (25.8%)	57 (61.3%)	93				

*The isolates were categorized based on the OD_{630} values (Stepanović *et al.*, 2007).

Table 2: Antibiotic resistance patterns of the identified biofilm forming bacteria from urinary catheter.

Isolates	AMP		AMC		AK		CN		E		CL		CFM		CRO		CIP		SXT		IPM		MEM	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
P. aeruginosa (n=34)	0	34	3	31	21	13	14	20	3	31	0	34	0	34	3	31	11	23	0	34	34	0	34	0
E. coli (n=10)	4	6	7	3	0	10	0	10	1	9	0	10	7	3	5	5	0	10	0	10	10	0	10	0
P. vulgaris (n=21)	0	21	0	21	0	21	0	21	0	21	0	21	0	21	0	21	0	21	0	21	21	0	21	0
K. pneumoniae (n=18)	0	18	0	18	7	11	11	7	0	18	1	17	7	11	4	14	11	7	0	18	18	0	18	0
S. aureus (n=10)	6	4	8	2	0	10	3	7	3	7	6	4	0	10	6	4	6	4	0	10	10	0	10	0

"S" denotes sensitive and "R" denotes resistant. The results are interpreted based on the inhibition zone diameter against tested antibiotics according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). The tested antibiotics are Ampicillin, AMP (10 µg), Amoxicillin/Clavulinic acid, AMC (30 µg), Amikacin, AK (30 µg), Gentamicin, CN (30 µg), Erythromicin, E (15 µg), Cephalexin, CL (30 µg), Cefixime, CFM (5 µg), Ceftriaxone, CRO (30 µg), Ciprofloxacin, CIP (5 µg), Trimethoprim/sulfamethoxazole, SXT (1.25/23.75 µg), Imipenem, IPM (10 µg) and Meropenem, MEM (10 µg).

Comparison of antibiotic sensitivity among biofilm-formers and non-biofilm-formers

When biofilm-forming uropathogenic bacteria were compared with non-biofilm-formers, the biofilm-formers showed significantly higher antibiotic resistance, trimethoprim/sulfamethoxazole (100% vs 82%), amoxicillin-clavulanic acid (81 vs 55%), cefixime (85% vs 55%), ceftriaxone (81% vs 45%), cephalexin (93% vs 55%), amikacin (70% vs 45%), ampicillin (89% vs 73%) and ciprofloxacin (70% vs 36%), with a *p*-value of less than 0.05. However, in the remaining antibiotics tested including erythromycin and gentamicin, there was no significant difference ($p>0.05$) in their action among biofilm and non-biofilm-formers (Table 3).

DISCUSSION

Catheter associated urinary tract infection is becoming one of the major hospital-associated infections (HAIs) globally. Longer duration of the urinary catheter in patients allows habituating bacteria in the device to form biofilms which eventually find their way to the urinary tract system and leads to infections (Donlan, 2002; Dougnon *et al.*, 2016; Awoke *et al.*, 2019). This

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Table 3: Comparison of antibiotic resistance pattern of biofilm-forming and non-biofilm forming bacteria.

Antibiotic used	Resistance by biofilm-formers (n) (%)	Resistance by non-biofilm formers (n) (%)	Odd ratio	95 % CI	*P* value	X^2
SXT	93 (100)	31 (82)	0.296	0.130-0.674	0.003*	8.887
E	86 (93)	35 (91)	0.950	0.232-3.883	0.943	0.005
CL	86 (93)	21 (55)	0.101	0.037-0.274	0.000*	24.961
AMP	83 (89)	28 (73)	0.337	0.127-0.895	0.025*	5.051
CFM	79 (85)	21 (55)	0.219	0.09-0.515	0.000*	13.158
CRO	75 (81)	17 (45)	0.194	0.086-0.441	0.000*	16.637
AMC	75 (81)	21 (55)	1.226	1.054-1.426	0.000*	18.099
AK	65 (70)	17 (45)	0.349	0.160-0.759	0.007*	7.291
CN	65 (70)	24(63)	0.738	0.334-1.634	0.454	0.562
CIP	65 (70)	14 (36)	0.251	0.114-0.556	0.000*	12.310
IPM**	0 (0)	0 (0)				
MEM**	0 (0)	0 (0)				

∗Biofilm-forming bacteria are more antimicrobial resistant compared with non-biofilm-forming ones ($p \leq 0.05$).
** Chi square tests not performed for "0" value.
Ampicillin, AMP (10 µg), Amoxicillin/Clavulinic acid, AMC (30 µg), Amikacin, AK (30 µg), Gentamicin, CN (30 µg), Erythromicin, E (15 µg), Cephalexin, CL (30 µg), Cefixime, CFM (5 µg), Ceftriaxone, CRO (30 µg), Ciprofloxacin, CIP (5 µg), Trimethoprim/sulfamethoxazole, SXT (1.25/23.75 µg), Imipenem, IPM (10 µg) and Meropenem, MEM (10 µg).

study provides data to quantify the biofilm forming ability of the isolated bacteria from urinary catheters and checked the status of the antibiotic resistance pattern of these biofilm formers.

All the catheters collected in this study were found culture positive with multiple bacterial species. The co-colonization by three different bacterial species in a single catheter was the highest. This type of multiple colonization in a single catheter was also recorded in previous studies (Macleod and Stickler, 2007; Verma *et al.*, 2016). We also observed the mixed bacterial community up to five species which also exhibited in another few studies (Kolter and Losick, 1998; Macleod and Stickler, 2007).

Among all colonized catheters, Gram-negative bacteria *P. aeruginosa* (29%), *E. coli* (24%), *K. pneumoniae* (16%) and Gram-positive *S. aureus* (16%) were predominant in this study. The high prevalence of *K. pneumoniae*, *E. coli*, *P. mirabilis*, *S. aureus* and *P. aeruginosa* were also reported from UTIs and CAUTIs by several studies from Ethiopia (Feleke *et al.*, 2018; Awoke *et al.*, 2019), Canada (Karlowsky *et al.*, 2011), Pakistan (Sabir *et al.*, 2017) and Saudia Arabia (Almalki and Varghese, 2020). We also observed the biofilm-forming capability of isolated bacteria quantitatively and 71% (93 out of 131) isolates exhibited biofilm-forming capacity *in vitro*.

The biofilm-forming capacity of these bacteria is similar or stronger than the isolates from previous studies. Awoke *et al.* (2019) reported that 80% of the total study isolates recovered from urinary catheterized inpatients were biofilm-formers where Sabir *et al.* (2017) also reported 73.4% (785/1070) of study isolates as biofilm-formers isolated from CAUTIs. Similar to our study, Murugan *et al.* (2016) also reported strong biofilm production by *S. aureus*, *E. faecalis* and *P. aeruginosa.* Moreover, we reported biofilm-forming capacity increased with multiple co-colonization as the bacteria that co-colonized with three or four mixed-species showed higher biofilm-forming capacity compared to two mixed-species. Cooperation among the mixed species was found to be increased in biofilm formation as all the co-colonizing species can help each other. Poor biofilm formers get benefited from this mutualistic relationship by utilizing the nutrients produced by one species and promote their growth inside the biofilm matrix (Rao *et al.*, 2020; Yuan *et al.*, 2020). Besides, it had been reported that co-culturing of *Lactococcus lactis* and *P. fluorescens* increased bacterial adhesion by up to 20,000- and 100-fold, respectively (Kives *et al.*, 2005).

Given to the complex microbial interactions, a mixed-species biofilm often achieves substantially more biomass than a monospecies biofilm without the need for extra nutrients such as in case of *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans* and *Paenibacillus amylolyticus* (Ren *et al.*, 2015). Similarly, previous studies also revealed the presence of mixed bacterial species in biofilm crystals observed from catheters (Kolter and Losick, 1998). Mixed community of *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae* and few other Gram-negative bacteria in crystalline biofilm on urinary catheter and their interaction in the biofilm also observed by other studies (Macleod and Stickler, 2007; Balasubramanian *et al.*, 2012).

Biofilm-producing bacteria is more resistant to antibiotics than the bacteria that do not form biofilms (Hashemzadeh *et al.*, 2020; Luo *et al.*, 2021). All the biofilm-forming isolates in our study were resistant to multiple antibiotics except for both carbapenems (meropenem and imipenem). These findings correlated with various other studies where multidrug-resistant pathogens were also reported (Nicolle, 2012; Dougnon *et*

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al., 2016; Awoke *et al.*, 2019). According to Prakash *et al.* (2013), most of the uropathogens were susceptible to carbapenem-group antibiotics imipenem and meropenem. This susceptibility was reported to be 92.26% for imipenem and 84.52% for meropenem (Prakash *et al*. 2013).

Among Gram-negative biofilm-forming bacteria, the most resistant pathogens in our study were *P. vulgaris* and *P. aeruginosa*, which showed maximum resistance to multiple antibiotics. This resistance pattern indicated that current antibiotics used to treat acute urinary tract infections seem ineffective in the eradication of isolated uropathogens. One of the possible reasons for high antibiotic resistance in biofilm formers can be the effect of selective antibiotic pressure on isolates. As all the isolates were collected from hospitalized postoperative patients with UTIs and the practice of frequent antibiotics use in a hospital-settings can exert selective antibiotic pressure on isolates leading to the acquisition of antibiotic resistance by nosocomial isolates (Kolář *et al.*, 2001).

Moreover, biofilm formers can also acquire drug resistance by delayed penetration through inactivation of antimicrobial molecules or limiting the diffusion of antimicrobial molecules through the polymer matrix of biofilm, decreasing antimicrobial uptake by slower the growth rate of biofilm associated cells, enzymatic transformation of biocidal agents, genetic adaptation and producing resistance phenotype and exchange of extrachromosomal DNA (Ehlers and Bouwer, 1999; Hausner and Wuertz, 1999; Roberts *et al.*, 1999; Donlan, 2002). It has been reported that when the planktonic bacterial cells form biofilm, they became resistant to antibiotics and to treat such infections, the concentration of antibiotics required to increase three- to four-times higher to achieve the bactericidal level depending on the species and drug combination (Gurung *et al.*, 2013).

In our study, the highest resistance was observed among biofilm-formers with trimethoprim/ sulfamethoxazole (100%), followed by erythromycin and cephalexin (93%), ampicillin (89%) and cefixime (85%). A study from Bangladesh reported the highest resistance against trimethoprim/ sulfamethoxazole, amoxicillin/ clavulanic acid, ceftazidime, ceftriaxone and cefixime by *E. coli* and *P. aeruginosa* (Hossain *et al.*, 2014). However, this study did not specify the biofilm-forming activities of the isolates. Karigoudar *et al.* (2019) also reported high resistance by biofilm-former against trimethoprim/ sulfamethoxazole. Sabir *et al.* (2017) also showed the highest resistance of ampicillin followed by ciprofloxacin among biofilm-formers. As trimethoprim/ sulfamethoxazole is one of the most frequently used antibiotics for UTI, unjudicial and overuse of the drugs may raise the resistance (Alam *et al.*, 2019).

The study data concluded that apart from both carbapenems, all the drugs exhibited less sensitivity against biofilm-formers. However, ciprofloxacin (36%), ceftriaxone (45%) and amikacin (45%) were effective against non-biofilm-formers. Excessive and inappropriate way of using antibiotics in hospital settings are the major factors which lead to the development of such antibiotic resistant bacteria. Furthermore, the biofilm-forming capability of these bacteria on the surface of medical devices (such as indwelling vascular catheters, cardiac pacemakers, prosthetic heart valves) that are installed for patient care increased the threat as biofilms protect the uropathogens from environmental stresses and it also leads to decreased susceptibility of the colonizing bacteria to the antibiotics. This will be more hazardous if infection prevention practices are not followed during patient care. Routine cleaning of the urethral meatus surface, using a small size catheter with good urinary drainage flow, using sterile and closed unobstructed urinary drainage, and cleaning the catheter and collecting system junction with chlorhexidine gluconate, a povidone-iodine solution, or a 70% isopropyl alcohol solution are effective infection prevention practices to reduce urinary catheter-related infections (Assadi, 2018).

There are some limitations in our study, such as the catheters were collected randomly without linking patients' profiles and hospital stays. We also acknowledged that the small sample size and absence of clinical records describing the types and severity of the urinary tract infections and profile of patients providing urinary catheters. However, our study provides data on antibiotic resistance patterns among catheter-associated biofilm-forming bacteria in two hospital settings of Bangladesh which prioritized the management guidelines for antibiotic use against such infections.

CONCLUSION

The diverse resistance pattern of uropathogenic bacteria highlights the importance of studying the pattern of urinary infection in every setting and the rational use of antibiotics in the management of such infections. In addition, rigorous hygiene practices and limited duration of catheterization should be implemented so that biofilm formation, as well as further emergence of antibiotic resistance can be reduced.

CONFLICT OF INTEREST

Disclose no conflict of interest.

ETHICAL APPROVAL

The study had been performed to fulfill the academic purpose and the approval to conduct the proposed study in the Department of Microbiology was taken. In this study, no patient data and specimens were being used, verbal consent was only taken from patients at the time of catheter collection.

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REFERENCES

Afroz, H., Fakruddin, M., Masud, M. R. and Islam, K. (2017). Incidence of and risk factors for hospital acquired infection in a tertiary care hospital of Dhaka, Bangladesh. *Bangladesh Journal of Medical Science* **16(3), 358-369.**

Alam, M. J., Asma, R., Chowdury, S. S. and Rahimgir, M. (2019). Sensitivity pattern of cefotaxime against common uropathogens *in vitro* in Dhaka, Bangladesh. *Drugs and Therapy Perspectives* **35(3), 145-149.**

Ali, S., Birhane, M., Bekele, S., Kibru, G., Teshager, L., Yilma, Y., Ahmed, Y., Fentahun, N., Assefa, H., Gashaw, M. and Gudina, E. K. (2018). Healthcare associated infection and its risk factors among patients admitted to a tertiary hospital in Ethiopia: Longitudinal study. *Antimicrobial Resistance and Infection Control* **7, 2.**

Almalki, M. A. and Varghese, R. (2020). Prevalence of catheter associated biofilm producing bacteria and their antibiotic sensitivity pattern. *Journal of King Saud University-Science* **32(2), 1427-1433.**

Assadi, F. (2018). Strategies for preventing catheter-associated urinary tract infections. *International Journal of Preventive Medicine* **9, 50.**

Awoke, N., Kassa, T. and Teshager, L. (2019). Magnitude of biofilm formation and antimicrobial resistance pattern of bacteria isolated from urinary catheterized inpatients of Jimma University Medical Center, Southwest Ethiopia. *International Journal of Microbiology* **2019, Article ID 5729568.**

Balasubramanian, A., Chairman, K., Singh, A. R. and Alagumuthu, G. (2012). Isolation and identification of microbes from biofilm of urinary catheters and antimicrobial susceptibility evaluation. *Asian Pacific Journal of Tropical Biomedicine* **2(3), S1780-S1783.**

CLSI, Clinical and Laboratory Standards Institute (2017). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Seventh Informational Supplement. CLSI Document M100-S27. Clinical and Laboratory Standards Institute, Wayne, PA.

Donlan, R. M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases* **8(9), 881-890.**

Dougnon, T. V., Bankole, H. S., Johnson, R. C., Hounmanou, G., Toure, I. M., Houessou, C., Boko, M. and Baba-Moussa, L. (2016). Catheter-associated urinary tract infections at a hospital in Zinvie, Benin (West Africa). *International Journal of Infection* **3(2), e34141.**

Ehlers, L. J. and Bouwer, E. J. (1999). Rp4 plasmid transfer among species of *Pseudomonas* in a biofilm reactor. *Water Science and Technology* **39(7), 163-171.**

Feleke, T., Eshetie, S., Dagnew, M., Endris, M., Abebe, W., Tiruneh, M. and Moges, F. (2018). Multidrug-resistant bacterial isolates from patients suspected of nosocomial infections at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Research Notes* **11(1), 602.**

Gurung, J., Khyriem, A. B., Banik, A., Lyngdoh, W. V., Choudhury, B. and Bhattacharyya, P. (2013). Association of biofilm production with multidrug resistance among clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from intensive care unit. *Indian Journal of Critical Care Medicine* **17(4), 214-218.**

Haque, M., Sartelli, M., McKimm, J. and Abu Bakar, M. (2018). Health care-associated infections - An overview. *Infection and Drug Resistance* **11, 2321-2333.**

Hashemzadeh, M., Dezfuli, A. A. Z., Nashibi, R., Jahangirimehr, F. and Akbarian, Z. A. (2020). Study of biofilm formation, structure and antibiotic resistance in *Staphylococcus saprophyticus* strains causing urinary tract infection in women in Ahvaz, Iran. *New Microbes and New Infections* **39, 100831.**

Hausner, M. and Wuertz, S. (1999). High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Applied and Environmental Microbiology* **65(8), 3710-3713.**

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology. 9th Edn. Lippincott Williams and Wilkins, USA.

Hossain M. D., Ahsan, S., Kabir, M. S. (2014). Antibiotic resistance patterns of uropathogens isolated from catheterized and noncatheterized patients in Dhaka, Bangladesh. *Tzu Chi Medical Journal* **26(3),127-131.**

Ige, O. K., Adesanmi, A. A. and Asuzu, M. C. (2011). Hospital-acquired infections in a Nigerian tertiary health facility: An audit of surveillance reports. *Nigerian Medical Journal* **52(4), 239-243.**

Iliyasu, G., Dayyab, F. M., Abubakar, S., Inuwa, S., Tambuwal, S. H., Tiamiyu, A. B., Habib, Z. G., Gadanya, M. A., Sheshe, A. A., Mijinyawa, M. S., Aminu, A., Adamu, M. S., Mande, K. M. and Habib, A. G. (2018). Laboratory-confirmed hospital-acquired infections: An analysis of a hospital's surveillance data in Nigeria. *Heliyon* **4(8), e00720.**

Jacobsen, S. M., Stickler, D. J., Mobley, H. L. T. and Shirtliff, M. E. (2008). Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clinical Microbiology Reviews* **21(1), 26-59.**

Karlowsky, J. A., Lagacé-Wiens, P. R. S., Simner, P. J., DeCorby, M. R., Adam, H. J., Walkty, A., Hoban, D. J. and Zhanel, G. G. (2011). Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. *Antimicrobial Agents and Chemotherapy* **55(7), 3169-3175.**

Karigoudar, R. M., Karigoudar, M. H., Wavare, S. M., Mangalgi, S. S. (2019). Detection of biofilm among uropathogenic *Escherichia coli* and its correlation with antibiotic resistance pattern. *Journal of Laboratory Physicians* **11(1), 17-22.**

Kives, J., Guadarrama, D., Orgaz, B., Rivera-Sen, A., Vazquez, J. and SanJose, C. (2005). Interactions in biofilms of *Lactococcus lactis* ssp. *cremoris* and *Pseudomonas fluorescens* cultured in cold UHT milk. *Journal of Dairy Science* **88(12), 4165-4171.**

Kolář, M., Urbánek, K. and Látal, T. (2001). Antibiotic selective pressure and development of bacterial resistance*. International Journal of Antimicrobial Agents* **17(5), 357-363.**

Kolter, R. and Losick, R. (1998). One for all and all for one. *Science* **280(5361), 226-227.**

Luo, Y., Yang, Q., Zhang, D. and Yan, W. (2021). Mechanisms and control strategies of antibiotic resistance in pathological biofilms. *Journal of Microbiology and Biotechnology* **31(1), 1-7.**

Macleod, S. M. and Stickler, D. J. (2007). Species interactions in mixed-community crystalline biofilms on urinary catheters. *Journal of Medical Microbiology* **56(11), 1549-1557.**

Maharjan, G., Khadka, P., Shilpakar, G. S., Chapagain, G. and Dhungana, G. R. (2018). Catheter-associated urinary tract infection and obstinate biofilm producers. *Canadian Journal of Infectious Diseases and Medical Microbiology* **2018, Article ID 7624857.**

Murugan, K., Selvanayaki, K. and Al-Sohaibani, S. (2016). Urinary catheter indwelling clinical pathogen biofilm formation, exopolysaccharide characterization and their growth influencing parameters. *Saudi Journal of Biological Sciences* **23(1), 150-159.**

Nicolle, L. E. (2012). Urinary catheter-associated infections. *Infectious Disease Clinics of North America* **26(1), 13-27.**

O'Toole, G., Kaplan, H. B. and Kolter, R. (2000). Biofilm formation as microbial development. *Annual Review of Microbiology* **54, 49-79.**

Potera, C. (2010). Antibiotic resistance: Biofilm dispersing agent rejuvenates older antibiotics. *Environmental Health Perspectives* **118(7), A288.**

Prakash, D. and Saxena, R. S. (2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. *International Scholarly Research Notices,* 2013.

Rao, Y., Shang, W., Yang, Y., Zhou, R. and Rao, X. (2020). Fighting mixed-species microbial biofilms with cold atmospheric plasma. *Frontiers in Microbiology* **11, 1000.**

Ren, D., Madsen, J. S., Sørensen, S. J. and Burmølle, M. (2015). High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. *The ISME Journal* **9(1), 81-89.**

Roberts, A. P., Pratten, J., Wilson, M. and Mullany, P. (1999). Transfer of a conjugative transposon, Tn5397 in a model oral biofilm. *FEMS Microbiology Letters* **177(1), 63-66.**

Sabir, N., Ikram, A., Zaman, G., Satti, L., Gardezi, A., Ahmed, A. and Ahmed, P. (2017). Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. *American Journal of Infection Control* **45(10), 1101-1105.**

Sharma, D., Misba, L. and Khan, A. U. (2019). Antibiotics versus biofilm: An emerging battleground in microbial communities. *Antimicrobial Resistance and Infection Control* **8, 76.**

Soltani, J., Poorabbas, B., Miri, N. and Mardaneh, J. (2016). Health care associated infections, antibiotic resistance and clinical outcome: A surveillance study from Sanandaj, Iran. *World Journal of Clinical Cases* **4(3), 63-70.**

Stepanović, S., Vuković, D., Hola, V., Di Bonaventura, G., Djukić, S., Cirković, I. and Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS* **115(8), 891-899.**

Storme, O., Saucedo, J. T., Garcia-Mora, A., Dehesa-Dávila, M. and Naber, K. G. (2019). Risk factors and predisposing conditions for urinary tract infection. Therapeutic *Advances in Urology* **11, 19-28.**

Verma, A., Bhani, D., Tomar, V., Bachhiwal, R. and Yadav, S. (2016). Differences in bacterial colonization and biofilm formation property of uropathogens between the two most commonly used indwelling urinary catheters. *Journal of Clinical and Diagnostic Research* **10(6), PC01-PC03.**

Yuan, L., Hansen, M. F., Røder, H. L., Wang, N., Burmølle, M. and He, G. (2020). Mixed-species biofilms in the food industry: Current knowledge and novel control strategies. *Critical Reviews in Food Science and Nutrition* **60(13), 2277-2293.**

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SUPPLEMENTARY INFORMATION

Table S1: The OD_{630} values of 131 isolates and interpretation of biofilm-forming capacity.

No.	Isolate	OD_{630}	Interpretation
1	*P. aeruginosa*-1	0.01	No Biofilm
2	*P. aeruginosa*-2	0.11	Weak Biofilm
3	*P. aeruginosa*-3	0.22	Moderate Biofilm
4	*P. aeruginosa*-4	0.24	Moderate Biofilm
5	*P. aeruginosa*-5	0.40	Strong Biofilm
6	*P. aeruginosa*-6	0.08	No Biofilm
7	*P. aeruginosa*-7	0.07	No Biofilm
8	*P. aeruginosa*-8	0.44	Strong Biofilm
9	*P. aeruginosa*-9	0.13	Weak Biofilm
10	*P. aeruginosa*-10	0.18	Weak Biofilm
11	*P. aeruginosa*-11	0.23	Moderate Biofilm
12	*P. aeruginosa*-12	0.07	No Biofilm
13	*P. aeruginosa*-13	0.39	Strong Biofilm
14	*P. aeruginosa*-14	0.39	Strong Biofilm
15	*P. aeruginosa*-15	0.29	Moderate Biofilm
16	*P. aeruginosa*-16	0.384	Strong Biofilm
17	*P. aeruginosa*-17	0.12	Weak Biofilm
18	*P. aeruginosa*-18	0.38	Moderate Biofilm
19	*P. aeruginosa*-19	0.39	Strong Biofilm
20	*P. aeruginosa*-20	0.18	Weak Biofilm
21	*P. aeruginosa*-21	0.24	Moderate Biofilm
22	*P. aeruginosa*-22	0.15	Weak Biofilm
23	*P. aeruginosa*-23	0.21	Moderate Biofilm
24	*P. aeruginosa*-24	0.18	Weak Biofilm
25	*P. aeruginosa*-25	0.16	Weak Biofilm
26	*P. aeruginosa*-26	0.17	Weak Biofilm
27	*P. aeruginosa*-27	0.16	Weak Biofilm
28	*P. aeruginosa*-28	0.16	Weak Biofilm
29	*P. aeruginosa*-29	0.18	Weak Biofilm
30	*P. aeruginosa*-30	0.15	Weak Biofilm
31	*P. aeruginosa*-31	0.17	Weak Biofilm
32	*P. aeruginosa*-32	0.29	Moderate Biofilm
33	*P. aeruginosa*-33	0.18	Weak Biofilm
34	*P. aeruginosa*-34	0.17	Weak Biofilm
35	*P. aeruginosa*-35	0.17	Weak Biofilm
36	*P. aeruginosa*-36	0.19	Weak Biofilm
37	*P. aeruginosa*-37	0.17	Weak Biofilm
38	*P. aeruginosa*-38	0.24	Moderate Biofilm
39	*E. coli*-1	0.05	No Biofilm
40	*E. coli*-2	0.05	No Biofilm
41	*E. coli*-3	0.21	Moderate Biofilm
42	*E. coli*-4	0.45	Strong Biofilm
43	*E. coli*-5	0.03	No Biofilm
44	*E. coli*-6	0.05	No Biofilm
45	*E. coli*-7	0.38	Strong Biofilm
46	*E. coli*-8	0.06	No Biofilm
47	*E. coli*-9	0.03	No Biofilm
48	*E. coli*-10	0.05	No Biofilm
49	*E. coli*-11	0.15	Weak Biofilm
50	*E. coli*-12	0.06	No Biofilm
51	*E. coli*-13	0.17	Weak Biofilm
52	*E. coli*-14	0.17	Weak Biofilm
53	*E. coli*-15	0.18	Weak Biofilm
54	*E. coli*-16	0.18	Weak Biofilm
55	*E. coli*-17	0.13	Weak Biofilm
56	*E. coli*-18	0.11	Weak Biofilm
57	*E. coli*-19	0.06	No Biofilm
58	*E. coli*-20	0.04	No Biofilm
59	*E. coli*-21	0.02	No Biofilm
60	*E. coli*-22	(0.04)	No Biofilm
61	*E. coli*-23	0.05	No Biofilm
62	*E. coli*-24	0.03	No Biofilm
63	*E. coli*-25	0.03	No Biofilm
64	*E. coli*-26	0.06	No Biofilm
65	*E. coli*-27	0.03	No Biofilm
66	*E. coli*-28	0.02	No Biofilm
67	*E. coli*-29	0.05	No Biofilm
68	*E. coli*-30	0.05	No Biofilm
69	*E. coli*-31	0.04	No Biofilm
70	*P. vulgaris*-1	0.07	No Biofilm
71	*P. vulgaris*-2	0.08	No Biofilm
72	*P. vulgaris*-3	0.16	Weak Biofilm
73	*P. vulgaris*-4	0.04	No Biofilm
74	*P. vulgaris*-5	0.13	Weak Biofilm
75	*P. vulgaris*-6	0.46	Strong Biofilm
76	*P. vulgaris*-7	0.19	Weak Biofilm
77	*P. vulgaris*-8	0.12	Weak Biofilm
78	*P. vulgaris*-9	0.19	Moderate Biofilm
79	*P. vulgaris*-10	0.19	Moderate Biofilm
80	*P. vulgaris*-11	0.20	Moderate Biofilm
81	*P. vulgaris*-12	0.13	Weak Biofilm
82	*P. vulgaris*-13	0.38	Strong Biofilm
83	*P. vulgaris*-14	0.15	Weak Biofilm
84	*P. vulgaris*-15	0.21	Moderate Biofilm
85	*P. vulgaris*-16	0.21	Moderate Biofilm
86	*P. vulgaris*-17	0.12	Weak Biofilm
87	*P. vulgaris*-18	0.19	Moderate Biofilm
88	*P. vulgaris*-19	0.194	Moderate Biofilm
89	*P. vulgaris*-20	0.13	Weak Biofilm
90	*P. vulgaris*-21	0.46	Strong Biofilm
91	*P. vulgaris*-22	0.13	Weak Biofilm
92	*P. vulgaris*-23	0.194	Moderate Biofilm
93	*P. vulgaris*-24	0.12	Weak Biofilm
94	*K. pneumoniae*-1	0.22	Moderate Biofilm
95	*K. pneumoniae*-2	0.12	Weak Biofilm
96	*K. pneumoniae*-3	0.07	No Biofilm
97	*K. pneumoniae*-4	0.15	Weak Biofilm
98	*K. pneumoniae*-5	0.10	Weak Biofilm
99	*K. pneumoniae*-6	0.03	No Biofilm
100	*K. pneumoniae*-7	0.21	Moderate Biofilm
101	*K. pneumoniae*-8	0.14	Weak Biofilm
102	*K. pneumoniae*-9	0.20	Moderate Biofilm
103	*K. pneumoniae*-10	0.12	Weak Biofilm
104	*K. pneumoniae*-11	0.39	Strong Biofilm
105	*K. pneumoniae*-12	0.11	Weak Biofilm
106	*K. pneumoniae*-13	0.20	Moderate Biofilm
107	*K. pneumoniae*-14	0.11	Weak Biofilm
108	*K. pneumoniae*-15	0.10	Weak Biofilm
109	*K. pneumoniae*-16	0.07	No Biofilm
110	*K. pneumoniae*-17	0.12	Weak Biofilm
111	*K. pneumoniae*-18	0.10	Weak Biofilm

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112	*K. pneumoniae*-19	0.14	Weak Biofilm
113	*K. pneumoniae*-20	0.11	Weak Biofilm
114	*K. pneumoniae*-21	0.10	Weak Biofilm
115	*S. aureus*-1	0.12	Weak Biofilm
116	*S. aureus*-2	0.10	Weak Biofilm
117	*S. aureus*-3	0.10	Weak Biofilm
118	*S. aureus*-4	0.08	No Biofilm
119	*S. aureus*-5	0.06	No Biofilm
120	*S. aureus*-6	0.05	No Biofilm
121	*S. aureus*-7	0.08	No Biofilm
122	*S. aureus*-8	0.11	Weak Biofilm
123	*S. aureus*-9	0.23	Moderate Biofilm
124	*S. aureus*-10	0.10	Weak Biofilm
126	*S. aureus*-11	0.01	No Biofilm
127	*S. aureus*-12	0.25	Moderate Biofilm
128	*S. aureus*-13	0.11	Weak Biofilm
129	*S. aureus*-14	0.11	Weak Biofilm
130	*S. aureus*-15	0.01	No Biofilm
131	*S. aureus*-16	0.10	Weak Biofilm
132	*S. aureus*-17	0.09	No Biofilm
135	ODc	0.10	No Biofilm

Table S2: Morphological, cultural, physiological and biochemical characteristics of isolates.

Parameter	*P. aeruginosa* isolates	*E. coli* isolates	*K. pneumoniae* isolates	*P. vulgaris* isolates	*S. aureus* isolates
Vegetative cell	Rod	Rod	Rod	Rod	Coccus
Cell arrangement	Single	Single	Single	Single	Cluster
Gram staining	Negative	Negative	Negative	Negative	Positive
Spore staining	Non spore former	Non spore former	Non spore former	Non spore former	Non spore former
Acid fast staining	Non-acid fast	Non-acid fast	Non-acid fast	Non-acid fast	Non-acid fast
Motility test	Motile	Motile	Non motile	Motile	Non motile
Growth on Cetrimide agar (Oxoid™ CM0579, UK)	Blue-green, circular, raised colony	Not observed	Not observed	Not observed	Not observed
Growth on MacConkey agar (Oxoid™ CM0007, UK)	Not observed	Bright pinky red, circular, flat colonies	Pink, circular, raised, mucoid colonies	Not observed	Not observed
Growth on Casein Enzyme Hydrolysate (CLED) agar (Oxoid™ CM0301, UK)	Not observed	Not observed	Not observed	Green-blue, circular, raised, slimy colony	Not observed
Growth on Mannitol Salt Agar (Himedia™ M118, India)	Not observed	Not observed	Not observed	Not observed	Circular, yellow, raised, smooth colony
Oxidase test	Positive	Negative	Negative	Negative	Negative
Catalase test	Positive	Positive	Positive	Positive	Positive
Tube coagulase test	Not done	Negative	Negative	Negative	Positive
Citrate utilization	Positive	Negative	Positive	Positive	Positive
Gelatin hydrolysis test	Positive	Negative	Negative	Negative	Positive
Casein hydrolysis test	Positive	Negative	Positive	Negative	Negative
Starch hydrolysis test	Negative	Negative	Positive	Negative	Positive
Voges-Proskauer test	Negative	Negative	Positive	Negative	Positive
Methyl red test	Negative	Positive	Negative	Positive	Negative
Nitrate reduction test	Positive	Positive	Positive	Positive	Positive
Triple sugar iron medium	Alkaline slant, acid butt without gas	Acidic slant, acidic butt, Gas +ve	Acidic slant, acidic butt, Gas +ve	Alkaline slant, Acidic butt, Gas +ve	Negative
H_2S production	Negative	Negative	Negative	Positive	Negative
Indole test	Negative	Positive	Negative	Positive	Negative
Urease test	Negative	Negative	Positive	Positive	Positive
Arginine dehydrolase	Positive	Negative	Negative	Negative	Positive
Ornithine decarboxylase	Negative	Positive (28 isolates) Negative (3 isolates)	Negative	Positive	Negative
Lysine	Negative	Positive	Negative	Negative	Not done

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			Fermentation test		
Arabinose	Negative	Positive	Positive	Negative	Negative
Cellobiose	Negative	Negative	Positive	Negative	Negative
DNase	Negative	Negative	Negative	Negative	Positive
Fructose	Positive	Positive	Negative	Not done	Positive
Galactose	Negative	Positive	Positive	Not done	Positive
Glucose	Positive	Positive	Positive	Positive	Positive
Lactose	Negative	Positive	Positive	Negative	Positive
Maltose	Negative	Positive	Positive	Negative	Positive
Mannitol	Positive	Positive	Positive	Negative	Positive
Mannose	Negative	Positive	Positive	Negative	Positive
Raffinose	Not done	Negative	Positive	Negative	Negative
Ribose	Positive	Positive	Not done	Negative	Positive
Salicin	Negative	Negative	Positive	Negative	Negative
Sucrose	Negative	Positive	Positive	Negative	Positive
Trehalose	Negative	Positive	Positive	Positive	Positive
Xylose	Negative	Negative	Positive	Positive	Negative

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