Study of prevalence biofilm forming bacteria and broad-spectrum antibiofilm activity as anti-quorum sensing agent of streptomyces secondary metabolites on urinary catheter infections

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ABSTRACT

Catheter-associated urinary tract infection is caused primarily by the multidrug-resistant biofilm forming bacteria of uropathogens. The prevention and successful treatment of infections is difficult when catheters are colonized by bacterial biofilm growth. There have been several efforts utilizing biocides in catheterizations, in attempts to inhibit all possible bacterial access to catheterized urethra. Nevertheless, it is clearly the case that such approaches are usually unsuccessful in the long run. Quorum quenching represents a new approach for control of infection for highly resistant microorganism. Therefore, there is a pressing need to find out natural Quorum Sensing inhibition compounds to overcome the resistance of the uropathogens. This unique concept of the antimicrobial control depends on reducing the burden of virulence and suppress the development of antibiotic resistance rather than killing the bacteria. In the present investigation, the target microorganisms were isolated from attached surface of urinary catheters which evaluated quantitatively and qualitatively using standard techniques. Altogether 100 isolates were collected. Antibiotic susceptibility assays results showed that most of the isolates were resistant to a wide range of beta lactam and non-beta lactam antibiotics. Results of biofilm forming on microtiter plate and sterile foley catheter reveled that these isolates possess a high capacity for biofilm formation on the both surface at the different degrees (strong, moderate and weak forming biofilm) and required the only a short contact time for attachment. An anti-biofilm secondary metabolites from streptomyces strain sdLi expressed broad-spectrum antibiofilm activity possibly by interfering with their QS systems against six different species producing strong biofilm and multi-drug resistance. The anti-biofilm of secondary metabolites strain sdLi against six species was examined on sterilized cover glasses and urinary catheter sections using light microscope and scanning electron microscopy respectively. Evidence is presented that sdLi streptomyces secondary metabolites showed broad-spectrum activity to attenuate the Quorum Sensing - dependent biofilm. The investigation of quorum-sensing control may lead to the development of novel antipathogenic drugs.

KEYWORDS: Urinary catheter, Broad-spectrum biofilm inhibition, Anti-quorum sensing and Streptomyces secondary metabolites.

INTRODUCTION

Catheter Associated Urinary Tract Infection (CA-UTI) is one of the most common nosocomial infections[1].

Several studies report an association of CA-UTI with increased mortality and prolonged length of stay in acute care facilities. For critical care unit patients, these associations are likely attributable to confounding by unmeasured variables with little, if any, mortality directly attributable to CA-UTI [2].

Contamination of the urine in the drainage bag can allow organisms to access the bladder through the drainage tube and the catheter lumen [3]. Many attempts have been made to employ biocides in the management of catheters to try to prevent bacterial access to the catheterized bladder by all possible routes. It has become clear, however, that, in the long term, these strategies are generally ineffective thus facilitate colonization by drug-resistant Gram-negative pathogens [4].

The longer the catheter remains in place the greater the variety of organisms that accumulate in the bladder. Long-term patients commonly become infected with mixed communities of mainly Gram-negative nosocomial species, such as *Psedomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumoniae.* These infections are difficult to eliminate with antibiotic therapy while the catheter remains in place[5]. Infected urine can, therefore, be flowing through catheters. Under these conditions, bacteria colonize the catheters, particularly the luminal surfaces, and form extensive biofilms [6], for example, found that these biofilms could colonize the whole length of the catheter lumen and be up to 400 cells deep, embedded in a polysaccharide gel-like matrix. The biofilm mode of growth is, of course, a basic survival strategy for bacteria and enables them to withstand a variety of environmental stresses [7]. In the case of bladder catheters there is evidence that bacteria in these biofilms, designated as sensitive to antibiotics by conventional laboratory procedures, survive the urinary concentrations of antibiotics produced by the standard [8]. Given the fact that biofilm formation is related to antibiotic resistance [9] ,The inhibition of quorum sensing (QS), or bacterial cell-to-cell communication, is one novel approach of an anti-pathogenic effect.

QS is a mechanism used by many bacteria to detect their critical cell numbers [10]. Cell densities are indicated by concentration of signal molecules that regulate common bacteria disease processes such as swarming, biofilm formation and secretion of virulence factors [11], thus this signaling pathway a novel and potential target for antimicrobial drugs that can act as QS inhibitors (QSI) to interfere with and attenuate QS-dependent bacterial pathogenicity without effecting on growth [12].

In the search for active secondary metabolites bacteria, Streptomyces attracts greater interest due to their ability to produce and secrete variety of secondary metabolites [13]. Several actinomycetes have been isolated from diverse natural resources. The biological function of actinomycetes mainly depends on the sources from which the bacteria are isolated. Natural resources forming mega biodiversity with strong selective pressure and intense microbial competition have gained well recognition as vital source for potential new-microbial agents in addition to anti-biofilm.

The purpose of the present study was to isolate, identified, determine the prevalence of biofilm forming bacteria and study their antibiotic susceptibility pattern from elderly patients undergoing long- term catheterization and to evaluate the effect of sdLi streptomyces secondary metabolites as anti-QS broad spectrum on sterilized cover glasses and urinary catheter sections using light microscope and scanning electron microscopy respectively.

MATERIAL AND METHODS

2.1 Sample collection and isolation of bacteria:

The study was performed at the University Kebangsaan Malaysia Medical Center and was conducted on three elderly patients who were suffering from acute vascular rejection Urinary Catheter named UCB and the others Benign Prostate Hyperplasia named UCC-D. All patients had been connected by urinary catheter for approximately continuous (14) days, the researcher has the permission from the hospital before the samples collection. The treatment of urinary catheters was performed according to [14, 15] with modifications. Briefly each urinary catheter were partitioned into four parts represented by (Eye-holes catheter(a); Lumen(b); Draining tube(c); Urine collection bag(d) and Urine(e)),parts were cut into 1cm thick discs (pieces) followed by successive washes of the inner surface with a solution of sodium chloride using the tip of micropipette and swabs for attached microorganisms , washing residues, swabs and pieces inoculated on to surface of nutrient agar (NA), macConkey agar (MA) and the plates were then incubated at 37 °C for24 – 48 h. Urine was taken from a catheter bag for each sample with a sterile syringe. Isolates were identified using biochemical tests based on (Bergeys Manual of Determinative Bacteriology) and API-20E test kit (Biom Merieus, France).

Fig. 3.1: The images shows isolation processing from urinary catheter samples

2.2 Antibiotic susceptibility:

In the present study antimicrobial susceptibility testing was done on Mueller- Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique. This method was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility of UTIs agent. The standard antibiotic comprised of ampicilin (10μg), chloramphenicol (30μg), gentamicin (10μg), kanamicin (30μg), novobiocin (30ug), penicillin G (10UI), streptomycin (10μg), and tetracycline (30μg).

2.3 Urinary catheter biofilm formation assay:

Biofilm formation on urinary catheter was investigated using the methods of [16] with some modifications. Foley catheter was cut into 1 cm pieces and immersed in each well of 96-well microtiter plate and LB broth supplemented with 20% urea containing test cultures to a final cell density of 1.5×10^8 CFU/mL. Samples were incubated at 37 $^{\circ}$C for 24 hours. After incubation the catheter pieces were washed twice with ddH$_2$O. Each piece was stained with 200 μL of 1% crystal violet solution in water for 20 min. The crystal violet was redissolved with ethanol and the optical densities were read using a microtiter-plate reader at 595 nm (Model 680, Bio-Rad). The average OD values were calculated for all tested strains and negative controls, the cut-off value (ODc) was established. It is defined as a three standard deviations (SD) above the mean OD of the negative control: ODc=average OD of negative control + (3×SD of negative control). Final OD value of a tested strain was expressed as average OD value of the strain reduced by ODc value (OD= average OD of a strain -ODc); ODc value was calculated for each microtiter plate separately. When a negative value was obtained, it was presented as zero, while any positive value indicated biofilm production. For easier interpretation of the results, strains were divided into the following categories:

Non biofilm producer (0) OD ≤ODc,
Weak biofilm producer (+ or 1) = ODc <OD ≤2×ODc,
Moderate biofilm producer (++ or 2) = 2×ODc <OD≤4×ODc,
Strong biofilm producer (+++or 3), 4×ODc <OD.

The experiment was performed in triplicate and the mean values were used. Six isolates identified as *Candida albicans*, *Proteus mirabilis*, *E.coli*, *Enterobacter cloacae*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were selected for anti-biofilm assays depending on strongest biofilm formation and multi-drug resistance.

2.4 Extraction of sdLi streptomyces secondary metabolites:

Secondary metabolites- producing Streptomyces strain sdLi (KX898581) was isolated from the saline lake sediment in Iraq. The growing conditions, period of incubation, fermentation medium and pH were optimized for the detection of the secondary metabolite compounds. These include growing Streptomyces strain sdLi in 1 litre of ISP2 (International Streptomyces Project) media (3 g of yeast extract, 3 g malt extract, 10 g glucose, supplemented with 4.5% NaCl, 0.5 ml glycerol) in 2 liter flasks incubated for 9 days at 29°C with shaking at 200 rpm. A modified method of [17]. Extraction step was used for the extraction of the bioactive metabolite compounds. The bacterial culture obtained was centrifuged at 10, 000 rpm for 15 min and cell-free supernatant was harvested and mixed with an equal volume of ethyl acetate and kept in rotary shaker at 200 rpm overnight. The solvent layer was harvested and then evaporated in a rotary evaporator in order to obtain the crude extracts,

to this, 100% of dimethyl sulfoxide (DMSO) was added to constitute a 50 mg/mL stock (w/v) from the sdLi crude extract and this stock was kept at 4 ^{o}C that was later used in the anti-biofilm assays.

2.5 *Effect of streptomyces secondary metabolites activity on biofilm formation:*

The anti-biofilm activity of the ethyl acetate crude extract from streptomyces strain sdLi for the six selected pathoginc isolates were determined using 96-well polystyrene microtiter plate, 1 cm sections pre-sterilized foley catheter and MBEC kit. The effect of sdLi crude extract on biofilm formation of isolates were assayed by quantification of the biofilm biomass as described in Urinary catheter biofilm formation assay. Overnight cultures of the isolates were sub-cultured in LB broth supplemented with 20% urea to a final cell density of 1.5 x 10^8 CFU/mL treated with 15 mg ml^{-1} sdLi crude extract or untreated (negative control), all incubated at 37°C for 24 h. The experiment was performed in triplicate and the mean values were used.

2.6 *Microscopic Analysis of Bacterial Biofilm Formation and Inhibition:*

The light microscopy analysis of biofilm was performed according to the methods of [18] with little modification. Briefly, 1% of overnight cultures of the untreated strongest biofilm forming isolates and treated with sdLi crude extract (15mg ml^{-1}) were added into 1 ml of fresh growth medium containing cover glass of 1 cm in 24-well MTP and incubated for 24 h. After incubation, the cover glasses were carefully removed and rinsed with distilled water to wash away the planktonic cells. The presumed biofilms that adhered on the cover glass were stained with 0.4% crystal violet (CV, Hi-Media, Mumbai, India) solution and then visualized under a light (Nikon Eclipse Ti 100, Tokyo, Japan).

Scanning electron microscopy (SEM) of catheter sections were performed according to the methods of [19] with minor modification. Briefly, Treatment with sdLi crude extract (15mg ml^{-1}) strongest biofilm forming isolates and the untreated were allowed to form biofilm on pre-sterilized sections of catheter (1cm in length) and the catheter were cut longitudinally into halves and immersed in the culture as earlier described in the quantification of biofilm formation assay. Following incubation on the culture, sections of the catheter were removed and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h and then washed overnight in phosphate buffer prior to being post fixed in Millonig's phosphate-buffered osmium tetroxide (1.0%) for 1 h. The samples were subjected to dehydration in a graded series of aqueous ethanol solutions (30 to 100%) and then critical point dried using liquid carbon dioxide. Finally, the samples were mounted on aluminum stubs sputtered with gold and examined in the scanning electron microscope.

2.7 *Statistical analysis:*

The Excel data analysis package was used to calculate mean, standard deviation of the mean. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.19. Significant differences (P<0.05). All the results were calculated from the mean of three replicate samples for each data point.

RESULTS AND DISCUSSION

Bacterial population in (NA, MA) agar plates showed to be varying density in all parts of three urinary catheters and urine samples (Table 1).

Table 1: Population density of bacteria isolated from urinary catheter (B, C, D), (a) Eye-holes catheter; (b Lumen; (c) Draining tube; (d) Urine collection bag; (e) Urine.

Urinary catheters	Parts	medium	
		NA	MC
(B)	a	1.46 x 10^3 (CFU/cm)	0.68 x 10^2 (CFU/cm)
	b	0.96 x 10^3 (CFU/cm)	0.59 x 10^2 (CFU/cm)
	c	0.78 x 10^3 (CFU/cm)	0.43 x 10^2 (CFU/cm)
	d	0.79 x 10^3 (CFU/cm)	0.50 x 10^2 (CFU/cm)
	e	0.79 x 10^3 (CFU/ml)	0.58 x 10^2 (CFU/ml)
(C)	a	1.48 x 10^3 (CFU/cm)	1.40 x 10^2 (CFU/cm)
	b	1.40 x 10^3 (CFU/cm)	1.30 x 10^2 (CFU/cm)
	c	0.89 x 10^3 (CFU/cm)	0.62 x 10^2 (CFU/cm)
	d	0.92 x 10^3 (CFU/cm)	0.89 x 10^2 (CFU/cm)
	e	0.99 x 10^3 (CFU/ml)	0.86 x 10^2 (CFU/ml)
(D)	a	1.40 x 10^3 (CFU/cm)	0.96 x 10^2 (CFU/cm)
	b	1.30 x 10^3 (CFU/cm)	0.90 x 10^2 (CFU/cm)
	c	1.00 x 10^3 (CFU/cm)	0.92 x 10^2 (CFU/cm)
	d	1.20 x 10^3 (CFU/cm)	0.95 x 10^2 (CFU/cm)
	e	1.09 x 10^3 (CFU/ml)	0.83 x 10^2 (CFU/ml)

Morphologically different bacterial colonies were observed on nutrient and MacConkey agar. Biochemical tests showed that 32 isolate were proteus and urease-positive , suggesting they will raise the urinary pH above 8 and produce catheter-blocking crystalline biofilms[19]. Total isolates were recovered from isolation agar plates and the results of microscopic were 100 isolates including 96 bacterial isolates and 4 yeast isolates have been successfully obtained from the three urinary catheters. Gram's staining and biochemical tests showed that 95% of the isolates were gram negative, the most common isolated pathogens were *Proteus mirabilis* 22 isolates (63%) followed by *E.coli* 5 isolates (14%), *Enterobacter cloacae* 4 isolates (11%), *Klebsiella pneumoniae* 3 isolates (9%), *Pseudomonas aeruginosa* 1 isolate (3%) for UC(B) while in UC(C and D) which represent the catheters to same disease were *Pseudomonas aeruginosa* 23 isolates (35%) showed the highest isolates followed by *E.coli* 16 isolates (24%), *Proteus mirabilis* 10 isolates (15%), *Enterobacter cloacae* 7 isolates (12%), *Klebsiella pneumoniae* 4 isolates (6%), *Candida albicans* 4 isolates (6%), *Bacillus* sp. 1 isolate (2%) (Table 2).

Table 2: Rate of isolation of microbes isolated from urinary catheter sections

Urinary Catheters	Isolates	No. of isolates Catheter	Urine	The total No. of isolates	(%) of isolates
UC(B)	*Proteus*	20	2	22	63
	E.coli	3	2	5	14
	Enterobacter	4	0	4	11
	Klebsiella	3	0	3	9
	Pseudomonas	1	0	1	3
UC(C&D)	*Pseudomonas*	22	1	23	35
	E.coli	16	0	16	24
	Proteus	7	3	10	15
	Enterobacter	7	0	7	12
	Klebsiella	4	0	4	6
	Candida	0	4	4	6
	Bacillus	1	0	1	2

These genera which isolated from all parts of the urinary catheter clarified that there was no great difference in the numbers and genera in all regions of the urinary catheter containing (Eye-holes catheter, Lumen, Draining tube, Urine collection bag).

Transitory microflora that originate from hospital personnel or from contact with other patients may represent antibiotic-resistant nosocomial strains, complicating treatment for these infections [20-22].

In our study the population density is equal in both catheters and urine but microbial diversity in the catheter is more than urine .It is possible that the bacteria that grow best on catheter surfaces are not the same as those that grow well in planktonic form in the urine or that bind well to urinary tract tissues. This may also indicate that the source of contamination was from extraluminal surface area in addition to contamination from intraluminal surface area catheter procedure, which is caused by bacteriuria, which indicates the presence of UTI.

Our data agree with Barford et al.'s study of 34 urology patients, which found more strains and genera on extraluminal than intraluminal surfaces[23] but contrast with those of [1]. Both ours and Barford's results are consistent with the hypothesis that the extraluminal surface is inoculated during catheter insertion. Colony-forming units increased along the urinary catheter surface from the catheter balloon to the urethra, this suggests extraluminal bacteria come from periurethral communities while intraluminal bacteria are introduced via the catheter or already inhabit the urine/bladder. The Enterobacteriacea family was the most common microorganisms isolated from urinary catheters. Increased use of antibiotics and immunosuppressive drugs such as corticosteroids are major factors contributing to higher frequency of fungal infections. Indwelling urinary catheters further favor the colonization of uropathogens by offering of a surface for the attachment of host cell binding receptors that are recognized by bacterial adhesions, thus enhancing microbial adhesion [24, 25].

Results from our analysis of genera composition on the catheters suggest that there may be a predictable succession in microbial communities over time and colonization is the first step towards symptomatic infection to which may evolve into biofilm in catheter segments.

Prevention of these infections has also proved to be problematic. Many attempts have been made to employ biocides in the management of catheters to try to prevent bacteria gaining access to the catheterized bladder by all possible routes. It has become clear, however, that, in the long term, these strategies are generally ineffective and, in certain cases (such as policies based on chlorhexidine), facilitate the colonization of the catheter drainage system and catheterized bladder by drug-resistant Gram-negative pathogens [26].

Antibiotic resistances of urinary catheter isolates were studied. (UCB) isolates showed highly resistant to ampicillin, novobiocin, penicillin (100%), kanamycin (97%), tetracycline (94%), chloramphenicol (91%), streptomycin (77%), and low levels of resistance to gentamycin, while the percentage of resistance to (UCC-D) isolates were highly resistant to ampicillin, penicillin (100%), novobiocin (94%) , tetracycline (61%),

streptomycin (53%), gentamicin (50%), and low levels of resistance to kanamycin (48%), chloramphenicol (47%) (Figure 1).
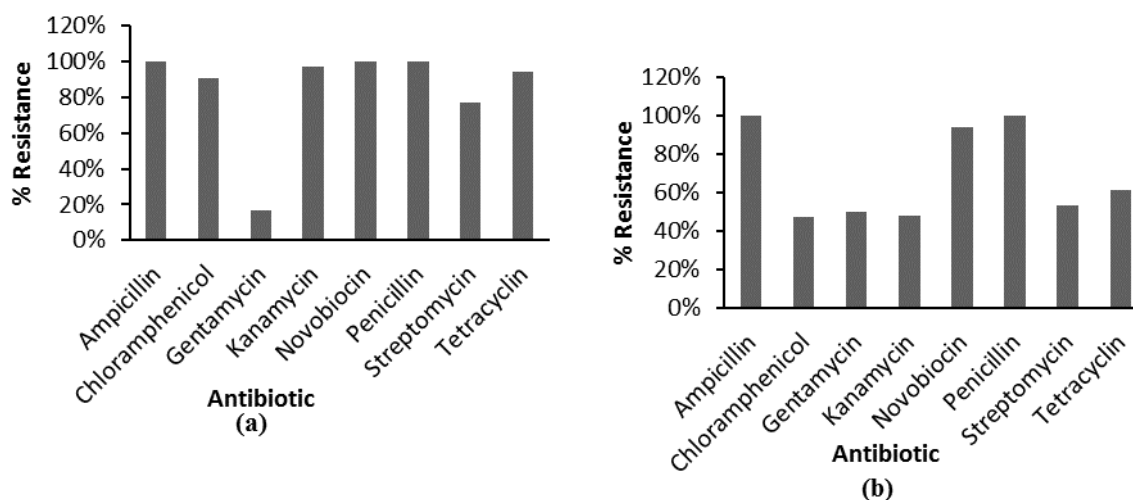

Fig. 1: Antimicrobial resistance of bacterial cultures isolated from (a) urinary catheter B, (b) urethral catheters C and D

Antibiotic resistance in UTIs and urinary catheter infections is increasing day by day, making it a major public health problem. So it is very important to determine the antibiotic resistance patterns in isolates for proper and accurate prescriptions.

Complications in UTIs have increased because of the prevalence of extended spectrum beta-lactamases (ESBL) producing bacterial pathogens which are also causing many management and epidemiological issues. Most of the ESBL are resistant to a wide range of beta-lactams and non-beta lactams. One of the major reasons for this high resistance can be co-expressed resistance mechanisms in the species of different pathogens isolated from patients of urinary tract infections [27]. In this study all the isolates showed multidrug resistance traits exhibited.

Biofilm formation plays an important role in the pathogenesis and development of pathogens biofilms CAUTI. Most aspects of the diagnosis, treatment, and prevention of CAUTI are influenced by the tenacity of biofilm-associated uropathogens. Previous studies have confirmed Long-term patients commonly become infected with mixed communities of mainly Gram-negative nosocomial species, Under these conditions, bacteria colonize the catheters, particularly the lumenal surfaces, and form extensive biofilms [6, 28].

Results of Biofilm production assay using Microtiter plate and pre-sterilized foley urinary catheter revealed that all of which possess a high capacity of adhesion which is the first step in the complex process of biofilm formation on different surfaces within short contact times, (only 24h) for attachment. This result is in agreement with the report of [5].

To better understand the findings, further analyses were conducted to classify isolates according to weak, moderate, and strong formation of biofilms. In the case of (UCB), of the 35 biofilm-forming isolates, 32% exhibited strong forming and 3% showed moderate forming, while with (UCC), of the 38 biofilm-forming isolates, 25% showed strong forming, 10% moderate forming and 3% showed weak biofilm forming. With (UCD) of the 28 biofilm-forming isolates, 26% exhibited strong forming and 2% showed moderate forming, as illustrated in (Table 3).

Table 3: Degree of biofilm formation in urinary catheter B, C and D

Isolates , %			
Catheters	Weak Biofilm	Moderate Biofilm	Strong Biofilm
Catheter (B)	0	3	32
Catheter (C)	3	10	25
Catheter (D)	0	2	26

To develop new drugs we have to understand the biological processes that interact between bacterial cells so this finding opens up the opportunity for a range of approaches we can use to block infections.

The results of the effect sdLi crude extract on biofilm development using urinary catheter surface suggests that the biofilm formation of the six pathogen isolates at 15 mg ml^{-1} sdLi crude extract were inhibited. Reduction in biofilm formation by 90% for the *Candida albicans*, 73% *Proteus mirabilis*, 57% *E. coli*, 78% *Klebsiella pneumoniae*, 92% *Pseudomonas aeruginosa* and 77% for *Enterobacter cloacae* were noted with

significant difference P< 0.05 (Figure 2). The similarity reduction was observed using 96-well polystyrene microtiter plates and MBEC kit assay (Figure 3).
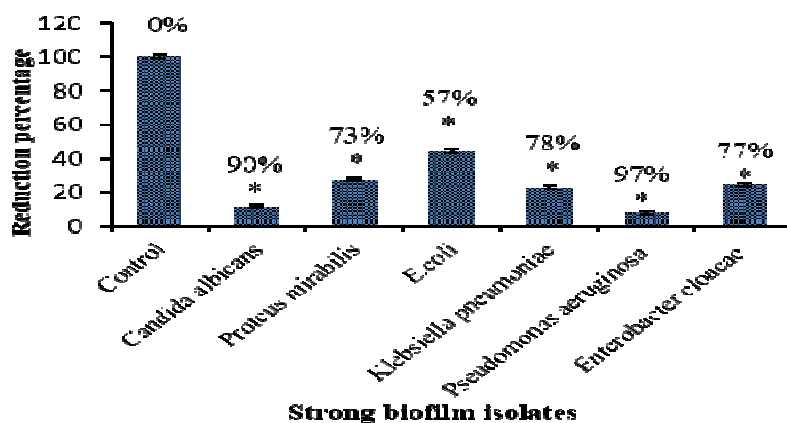

Fig. 2: Urinary catheter biofilm assay. Reduction of biofilm forming for six pathogens isolates using 15 mg ml-1 sdLi crude extract were noted with significant difference P< 0.05 (The results were reproduced in three experiments and the error bars indicate standard deviation)
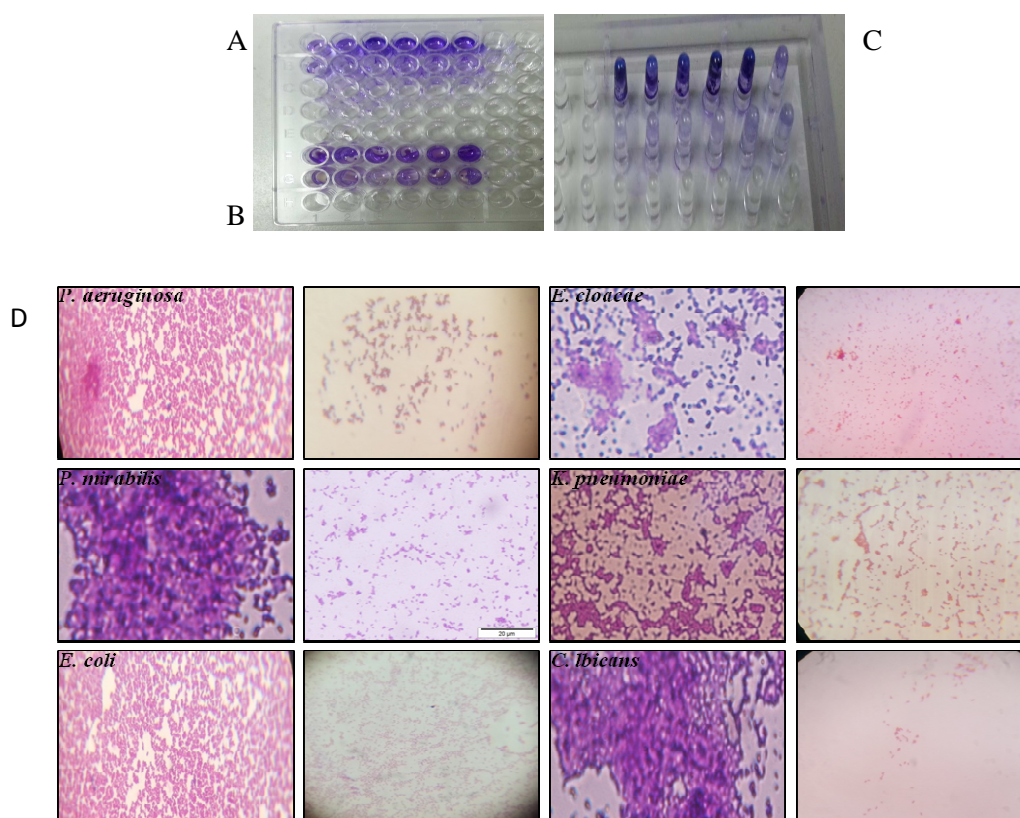

Fig. 3: Biofilm development of the six isolates grown in the absence and presence of 15 mg ml-1 sdLi crude extract showing inhibitory activity; A: polystyrene microtiter plate. B: urinary catheter sections. C: MBEC kit. D: light microscopic images.

An interesting additional mechanism in the development of biofilms is the characteristic architecture of the biofilm [30]. Direct microscopic observations of biofilms is known to provide valuable information of the quantifying the biofilm biomass for treated and untreated cells, therefore SEM microscopy analyses were performed.

The light microscopy analysis and scanning electron microscopy images (Figure.3, 4) for untreated isolates showed that the selected identified isolated genus formed a dense and interconnected networks of cellular multilayer's that were surrounded and enveloped by a protective matrix on cover glass and inner surface of foley

urinary catheter sections while for the treated isolates the both surface showed high reduction of biofilm for all the isolates.
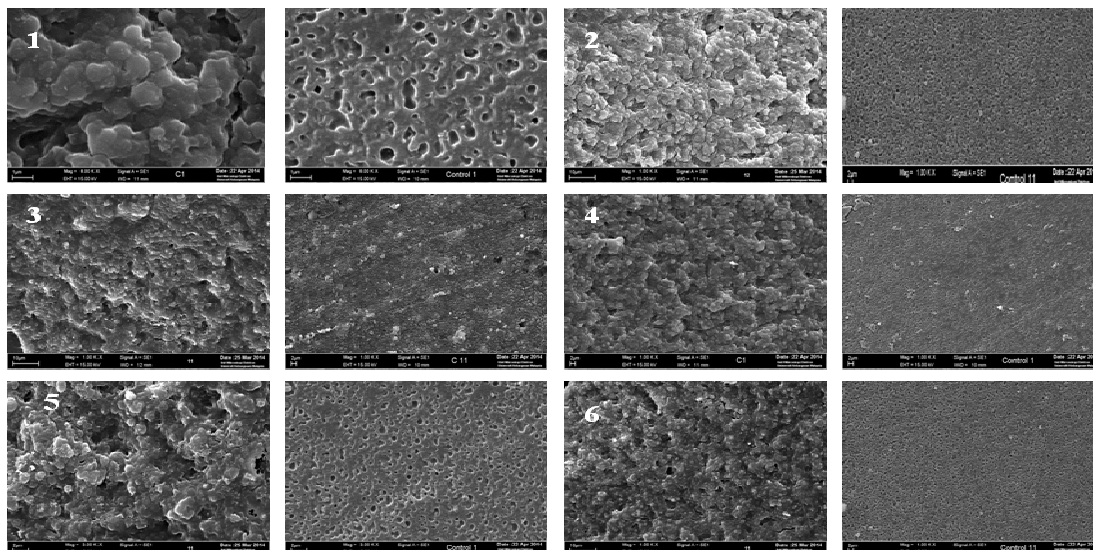

Fig. 4: Growth of bacterial biofilms as observed from the microscopic images of scanning electron microscope (SEM): untreated cells (controls) and treated cell with sdLi crude extract, *C. albicans* 1, *P. mirabilis* 2, *E. coli* 3, *P. aeruginosa* 4,: *E. cloacae* 5,: *K. pneumonia* 6.

Urinary catheter rubber surfaces are prone to deterioration and may develop surface cracks where bacteria can accumulate, thus a thick coating of biofilms was observed in controls to provide cells resistance to antimicrobial agents, whereas a visible reduction in numbers of microcolonies was observed in the biofilms of all the treated isolates. On the other hand, sdLi crude extract deteriorated the architecture of the biofilm as was more evident from SEM analysis. Our results are consistent with previous reports of [31] with furanone of *D. pulchra* and Bjarnsholt, [32] with garlic, in which a significant reduction in biofilm morphology and a reduction thickness of *P. aeruginosa* was observed without effective on cells growth.

Since the effect of sdLi crude extract was loss the architecture of the biofilm with uninfluenced plankton cells there is growing evidence that sdLi crude extract have broad-spectrum activity as anti-quorum sensing to attenuate the Quorum Sensing (QS) - dependent biofilm which have the role in the maturation of bacterial biofilms [33].

SdLi (KX898581) crude extract have been reported in the previous study to possess anti- biofilm activity and anti-QS properties of the human pathogens *P. mirabilis* [29], in our data sdLi crude extract showed significant ($p < 0.05$) broad-spectrum anti-biofilm activity against the six different pathogens when treated with 15 mg ml^{-1} sdLi crude extract.

Conclusion:

The study revealed that catheter-associated UTI in University Kebangsaan Malaysia Medical Center is caused by strong positive variety of multi-drug resistance gram negative bacteria and yeasts which can be able to produce strong biofilm on microtiter plate, cover glass and urinary catheter surfaces. The streptomyces strain sdLi crude extract used in this study demonstrated a broad-spectrum activity as anti-quorum sensing to attenuate the Quorum Sensing (QS)-dependent biofilm and can be a good candidate agent in combating urinary catheter infection.

Recently, it has been suggested to develop therapeutics that attack the bacterial virulence rather than kill bacteria. Such substances are called "antipathogenic" and are believed to minimize the development of antibiotic resistance.

Probably, mechanism of quorum-sensing repression of biofilm formation in gram-negative bacteria might be a base for drug development aimed at controlling biofilm-associated infection.

A new alternative strategy of antibacterial therapy needs which includes the construction of drugs acting directly against bacterial pathogenicity (antipathogenicity drugs) as indicated by the results of the current study. This strategy makes it possible to avoid a wide distribution of antibiotic-resistant pathogenic bacteria and the formation of biofilms. Specifically, cell-density-dependent gene regulation (quorum-sensing) in bacteria has been proposed as a potential target.

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