Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 3, 2014

Original Article

EFFECTS OF ANTIBIOTICS UPON QUORUM SENSING REGULATED CHARACTERS: A PROPITIOUS SCHEME AGAINST DEVICE ASSOCIATED INFECTIONS

PRASANTH RATHINAM[1], PRAGASAM VISWANATHAN[1*]

[1]Renal Research Laboratory, Bio Medical Research Centre, School of Biosciences & Technology, VIT University, Vellore, India.
Email: pragasam.v@vit.ac.in / pvishvanath@yahoo.com

Received: 08 Jan 2014, Revised and Accepted: 21 Apr 2014

ABSTRACT

Objective: Catheter associated urinary tract infection (CAUTI) is considered as one of the prevalent nosocomial infection, where the severity directly correlates with the period of catheterization. Strategies like improved biomaterials and anti-microbial coatings lack enough evidences to be considered as an effective scheme against CAUTI and retain the risk of developing resistance. Since most of the virulence factors in pathogens are under quorum sensing (QS) switch, QS antagonists are favored to be an effective policy against CAUTI. Hence, the aim of this study was to evaluate the anti-QS activity of sub-inhibitory concentrations (SIC) of selected antibiotics.

Methods: Five different antibiotics; Nalidixic acid (NAL), Ampicillin (AMP), Amoxicillin (AMX), Cefuroxime (CXM), Co-trimoxazole (COT) were selected and Azithromycin (AZM) was used as the positive control. The SIC were determined against *P. aeruginosa* PAO1 and 2 clinical isolates of *Pseudomonas* sp. (PA-1 and PA-2). All the test compounds were quantified for the reduction in expression of the QS regulated behaviors.

Results: All the antibiotics under investigation exhibited reduction in the production of QS controlled factors in the test organisms. However, CXM at 20 µg/mL, 36 µg/mL and 32 µg/mL displayed significant reduction in the quorum regulated genes of PAO1, PA-1 and PA-2 respectively in comparison with the positive control AZM. While the other test compounds failed to generate comparable results.

Conclusion: It has been surmised that cephalosporin antibiotic - CXM effectively subdued QS regulated factors and could be explored as a superior prospect against CAUTI, either as a coating or as a wash solution.

Keywords: Catheter associated urinary tract infection, Cefuroxime, Quorum sensing inhibition, Sub - inhibitory concentration.

INTRODUCTION

The urinary tract is the most common site of nosocomial infection (30% - 40%) and is always associated with indwelling catheters. The risk of developing bacteriuria from a catheter increases by 3% - 6% per day and become 100% within 30 days [1]. Because of the high incidence rate, catheter associated urinary tract infection (CAUTI) is responsible for an enormous burden of morbidity, mortality and increased health care costs [2]. Hence several approaches have been adopted to limit the colonization of devices, such as, strict hygienic practices for health care personals and environment. However, it should be noted that routine disinfection in hospital environment is an element of controversy [3].

Though most experts recommend treating CAUTI with 5-10 days of targeted antibiotic therapy [4, 5], the treatment usually promote the antimicrobial resistance in patients' flora and recurrent bacteriuria [6, 7]. Likewise, many strategies were implemented, but these strategies achieved only short term suppression of bacteriuria [2, 8, 9, 10, 11] and merely delayed the onset of bacteriuria in chronically catheterized situations [12, 13]. Catheters with improved biomaterials and antimicrobial coatings have captured researcher's attention [14, 15]. Impregnating urinary catheters with anti-microbial agents like silver ions and nitrofurazone found to be effective only in short term catheterization settings [2, 14] and leads to the emergence of antimicrobial resistance and adverse drug effects in patients [6, 16, 17]. Thus currently, no proven effective strategies exist for the prevention of CAUTI in subjects with long term catheterization [13].

Considering the fact that virulence factors (VFs) are central to the pathogenesis of infections [13, 15, 18], novel methods like hindering virulence factor production might assist in prevention of CAUTI [19]. Unlike antimicrobials, this strategy would not impart selective pressure to microbes, thus preventing development of resistance. Attenuation of pathogenic microbes would not alter the natural microbiome of host and work optimally to prevent infection than curing an established infection [19]. Inhibition of quorum sensing (QS), a mechanism which utilizes small molecules to regulate the production of VFs in microbes [20], recognized as a possible target for the prospected scheme. Modeling catheters which are either coated or irrigated with anti-QS compounds might attenuate the VFs in pathogens and can be used as an effective strategy to prevent device associated infections [19]. Researchers have been focusing on developing chemical antagonists which can interfere with bacterial virulence by hampering the QS circuit [19, 21].

P. aeruginosa PAO1 is one of the major candidates to study the effect of quorum sensing inhibition (QSI) as an anti-infective strategy [22] due to the expanded understanding about its pathogenic mechanisms [23, 24]. *P. aeruginosa* is the third most important organism associated with CAUTI in immuno compromised patients. [25]. Expression of diverse pathogenetic traits in *P. aeruginosa* is controlled by at least 2 QS systems, *lasI/lasR* [26] and *rhlI/rhlR* [27] which is arranged hierarchically. Hence QS interference has been an important anti-infective strategy against *P. aeruginosa* infections [19, 27]

The present study evaluated the anti-QS effect of sub-inhibitory concentration (SIC) of antibiotics upon the expression of VFs of isolates from CAUTI patients. The selected antibiotics were used against urinary tract infection (UTI) and their SIC levels were estimated against the test organisms. The effects of these antibiotics upon the VFs of the foresaid organisms at the SIC were also analyzed to compare the anti-QS effects. Our results offer new and diverse implications of antibiotics for the treatment of device associated infections.

MATERIALS AND METHODS

Chemicals

The antibiotics and other microbial media used in the study were purchased from Himedia laboratories PVT Ltd. (Bangalore, India). All the other chemicals, solvents and reagents used for the quantification of VFs were procured from Sisco Research Laboratories PVT Ltd. (Mumbai, India).

Bacterial strains used in this study

Two clinical isolate of *Pseudomonas* sp. (PA-1 and PA-2) were collected from CAUTI patients and used along with a reference strain

P. aeruginosa PAO1 (MTCC NO. 2453, IMTECH, Chandigarh, India). The clinical isolates were isolated from patients undergoing permanent catheterization due to cystitis and bladder thickening. All these microbes were cultured aerobically in Luria-Bertani (LB) broth (pH-7.2) with 120 rpm/min agitation in a rotatory shaker at 37°C for overnight. For experimental purpose all the microbes were sub-cultured using the same medium to reach a final OD of 0.4 at 600 nm.

Determination of SIC levels of antibiotics against the selected microbes

Antibiotics used against UTI, Nalidixic acid (NAL), Ampicillin (AMP), Amoxicillin (AMX), Cefuroxime (CXM) and Co-trimoxazole (COT) were selected and Azithromycin (AZM) was used as the positive control for this study. All the antibiotics were dissolved and diluted using appropriate solvents and diluents, as per the Clinical and Laboratory Standards Institute (CLSI), USA (2006) guidelines [28] to attain the concentrations ranging from 1 µg/mL-120 µg/mL. The SIC of antibiotics was determined using macro-dilution method as per the guidelines of CLSI [28].

Concentrations below the minimum inhibitory concentration (MIC) were considered as SIC and all the other experiments in the present study were performed at SIC of the antibiotics.

Pyocyanin quantification assay

Antibiotics at their appropriate SIC were added in 5 mL of LB broth containing 1% of early stationary phase grown test organisms (OD 0.4 at 600 nm) and incubated at 37°C up to late stationary phase of growth. AZM at 2 µg/mL was used as a positive control along with a growth control (without any test compound). After incubation, the cell free supernatants of test organisms (with or without test compounds) extracted with 3 mL of chloroform and re-extracted using 1 ml of 0.2N HCl to get a pink to deep red solution. The absorbance of the solution was read spectrophotometrically at OD_{520} (ELx - 800, Biotek) [29].

Pyoverdine quantification assay

The pyoverdine quantification was adopted from Cox and Adams (1985) [30] and the relative concentration of pyoverdine was measured using a fluorescence spectrometer (Fluorescence Spectrometer FP-8200, Jasco). The concentration was expressed as relative florescence units per ml of culture supernatant. To eliminate the possibility of false positive results due to an indirect effect of pH, pH was checked throughout the assay period [31].

Effect of antibiotics upon biofilm formation

The effect of antibiotic SIC levels upon biofilm formation was studied through quantifying the biofilm biomass through microtitre plate (MTP) assay [32]. The biofilm biomass was quantified by measuring the intensity of crystal violet (CV) in ethanol at OD_{650} using a UV-visible spectrophotometer (ELx-800, Biotek).

Effect upon Extracellular polymeric substances (EPS) formation

Test organisms were allowed to form biofilms over glass slides immersed in appropriate media (2X2 cm) with or without the antibiotics by incubating at 37°C and allowed to grow till late stationary phase. After the incubation the EPS was quantified by total carbohydrate assay [29]. In brief, the glass slides were washed in 0.9% of NaCl and incubated with an equal volume of 5% phenol and 5 volumes of concentrated H_2SO_4. The mixture was incubated for an hour in dark and the absorbance was measured at 490nm using a UV-visible spectrophotometer (ELx-800, Biotek) [29].

Cell density analysis

To confirm the effect of SIC levels of antibiotics upon test organisms' proliferation, cell density was thoroughly analyzed in every assay. Briefly, 1% of the overnight culture of the test organisms were inoculated and incubated at 37°C to reach late stationary phase. After incubation OD_{600} was analyzed using a UV-[pvisible spectrophotometer (ELx-800, Biotek) and the viable cells were counted through spread plate method on a nutrient agar plate. All measurements were performed with 1/10 dilutions to verify the results [31].

Statistical analysis

All experiments were performed independently (n=6) and the data was represented as mean values ± Standard Deviation (SD). The % inhibition of virulence factors by the antibiotics was measured using the formula: % inhibition = 100-(a×100)/b where, 'a' is the respective virulence factor expression with antibiotics and 'b' is that of growth control. The difference between the control and the test were analyzed using one way ANOVA with a p value of 0.05 being significant using GraphPad Prism 5.

RESULTS

Determination of SIC of the antibiotics

Five antibiotics, which are conventionally used against UTI, were selected for the study. Azithromycin (AZM) was used as the positive control. MIC of all the antibiotics was assessed for all the test organisms (PAO1, PA-1 and PA-2) and represented in Table 1. Concentrations below the MIC level considered as SIC and all the further assays were carried out in the SIC of these selected antibiotics.

Table 1: MIC of antibiotics against the test organisms

Antibiotics	*P. aeruginosa* PAO1 (µg/mL)	Clinical Isolates (µg/mL)	
		PA-1	PA-2
CXM	102	112	98
AMX	46	82	50
COT	32	66	36
NAL	34	52	38
AMP	30	58	28
AZM	82	108	88

Effect of antibiotics upon pyocyanin production

In order to analyze the pyocyanin production, the cells were cultivated in the presence and the absence of the test compounds (Table 2, 3 and 4). A significant decrease in pyocyanin production by PAO1 was observed to the level of 80% by CXM (20 µg/mL) which was on par with AZM (2 µg/mL). NAL (1µg/mL) reduced 74% pyocyanin production in PAO1. CXM (36 µg/mL) produced 65% reduction in pyocyanin production by PA-1, while AZM (3 µg/mL) reduced it to a factor of 76.6%. In PA-2, CXM (32 µg/mL) inhibited the pyocyanin production by a factor of 76.36% while with the positive control (2 µg/mL) the reduction was 81%.

The other antibiotics in the investigation reduced the pyocyanin production but the reduction rate was not comparable with that of the positive control.

Effect of antibiotics upon pyoverdine production

The effect of SIC levels of antibiotics upon pyoverdine production by PAO1, PA-1 and PA-2 is represented in Table 2, 3 and 4 respectively. CXM (20 µg/mL) reduced 93.7% of pyoverdine production by PAO1 which was in equivalence with 94.96% by AZM (2 µg/mL). AZM reduced the pyoverdine production of both, PA-1 and PA-2 by a factor of 99.76% (3 µg/mL) and 99.42% (2 µg/mL) respectively. Similarly CXM (36 µg/mL) reduced the pyoverdine production by

81.60% in PA-1 while in PA-2; it was 82.45% at 32 µg/mL. Among the other antibiotics, NAL (14 µg/mL) inhibited 79.0% of the pyoverdine production by PA-1 where as in PA-2 COT (12 µg/mL) reduced 76.0%. The culture supernatants retained a pH of 7.0, regardless of the amount of the solvent or diluents added along with the test compound (data not shown).

Effect of antibiotics upon biofilm formation

Amoxicillin (5 µg/mL) significantly inhibited 68.38% of PAO1's biofilm production which was in par with that of AZMs' (2 µg/mL) 74.70%. Among the other antibiotics, COT (3 µg/mL), NAL (1 µg/mL) and CXM (20 µg/mL) inhibited the biofilm formation by the factor of 40.17%, 40.16% and 38.46% respectively. CXM (36 µg/mL), AMX (14 µg/mL) and NAL (14 µg/mL) dislodged the biofilm formation by 68.50%, 63.74% and 65.57% respectively in PA-1, which was in par with that of 77.25% of AZM (3 µg/mL). AMX at 12 µg/mL and CXM at 32 µg/mL effectively dislodged the biofilm biomass by 51.72% and 48.76% respectively. AZM (2 µg/mL) reduced the biofilm formation by 73.39% in PA-2. The biofilm formation by the test organisms under the influence of the test compounds are represented in Table 2, 3 and 4.

Effect of antibiotics upon EPS formation

Cefuroxime at 20 µg/mL, 36 µg/mL and 32 µg/mL inhibited EPS production by 71.67%, 69.92% and 65.13% correspondingly in PAO1, PA-1and PA-2. But AZM (2 µg/mL) inhibited the EPS production in PAO1 by 83.37%. Among the clinical isolates; PA-1 and PA-2, AZM at 3 µg/mL and 2 µg/mL inhibited the EPS production by 79.29% and 80.98% respectively. The effect of various antibiotics at SIC levels were represented in Table 2, 3 and 4.

Cell density analysis

During every assay the cell density was analyzed and compared with the growth control and expressed as percentage growth/mL of culture. The obtained data are given in Table 2, 3 and 4.

Table 2: Effects of antibiotics upon virulence factors produced by *P. aeruginosa* PAO1

Antibiotics (µg/ml)	Pyocyanin [a] production	Pyoverdine [b] production	Biofilm [c] formation	EPS [d] production	Cell density [e] (%/mL)
Growth ctrl	0.12 ± 0.003	304.5 ± 0.02	1.17 ± 0.01	0.6 ± 0.025	100.00
CXM (20)	0.03 ± 0.001 [ns]	19.9 ± 0.01 [ns]	0.72 ± 0.03**	0.17 ± 0.013 [ns]	98.98 ±1.2
AMX (5)	0.08 ± 0.001***	193.0 ± 0.02***	0.37 ± 0.06 [ns]	0.48 ± 0.015***	99.27 ± 1.8
COT (3)	0.06 ± 0.004***	122.4 ± 0.02***	0.70 ± 0.01**	0.29 ± 0.01**	98.45 ± 1.3
NAL (1)	0.039 ± 0.003*	77.3 ± 0.01***	0.71 ± 0.02**	0.44 ± 0.03***	99.67 ± 0.94
AMP (4)	0.09 ± 0.002***	205.9 ± 0.002***	0.90 ± 0.017***	0.43 ± 0.01***	98.13 ± 1.7
AZM (2)	0.02 ± 0.007	15.3 ± 0.03	0.296 ± 0.022	0.1 ± 0.029	98.98 ± 2.1

[a] Pyocyanin production was expressed as OD_{520} after the incubation with 0.2N HCl. [b] Pyoverdine production was expressed as relative florescent units at 465nm (excitation λ=405nm) per mL of the culture. [c] Biofilm production was expressed as OD_{520} after incubating with crystal violet. [d] EPS production was expressed as OD_{490} after 5% Phenol-H_2SO_4 (1:5 v/v) treatment. [e] Cell density was expressed as % growth per mL of the culture in comparison with the growth control. ns- non significant: * significant at p<0.05: **significant at p<0.01 and ***significant at p<0.001.

Table 3: Effects of antibiotics upon virulence factors produced by clinical isolate PA-1

Antibiotics (µg/ml)	Pyocyanin [a] production	Pyoverdine [b] production	Biofilm [c] formation	EPS production [d]	Cell density [e] (%/mL)
Growth ctrl	0.15 ± 0.0025	476.70 ± 16.06	2.73 ± 0.083	1.21 ± 0.005	100.00
CXM (36)	0.05 ± 0.0025 [ns]	87.67 ± 4.36*	0.86 ± 0.027*	0.36 ± 0.028 [ns]	99.32 ± 1.9
AMX (14)	0.09 ± 0.002***	273.87 ± 7.87***	0.99 ± 0.045**	1.10 ± 0.0145***	99.45 ± 2.01
COT (18)	0.08 ± 0.0012**	117.23 ± 9.01**	1.52 ± 0.040***	0.55 ± 0.036*	98.93 ± 1.3
NAL (14)	0.06 ± 0.006*	99.53 ± 1.69**	0.94 ± 0.031**	0.57 ± 0.018*	97.96 ± 1.3
AMP (10)	0.07 ± 0.0023**	420.23 ± 1.23***	1.93 ± 0.046***	0.91 ± 0.009**	99.3 ± 2.1
AZM (3)	0.03 ± 0.0025	34.80 ± 5.96	0.621 ± 0.023	0.25 ± 0.032	98.79 ± 1.7

[a] Pyocyanin production was expressed as OD_{520} after the incubation with 0.2N HCl. [b] Pyoverdine production was expressed as relative florescent units at 465nm (excitation λ=405nm) per mL of the culture. [c] Biofilm production was expressed as OD_{520} after incubating with crystal violet. [d] EPS production was expressed as OD_{490} after the 5% Phenol-H_2SO_4 (1:5 v/v) treatment. [e] Cell density was expressed as % growth per mL of the culture in comparison with the growth control. ns- non significant: * significant at p<0.05: **significant at p<0.01 and ***significant at p<0.001.

Table 4: Effects of antibiotics upon virulence factors produced by clinical isolate PA-2

Antibiotics (µg/ml)	Pyocyanin [a] production	Pyoverdine [b] production	Biofilm [c] formation	EPS [d] production	Cell density [e] (%/mL)
Growth ctrl	0.11 ± 0.0015	427.37 ± 8.63	2.03 ± 0.042	1.26 ± 0.027	98.97 ± 1,9
CXM (32)	0.03 ± 0.009 [ns]	75 ± 3.44*	1.04± 0.044**	0.44 ± 0.037*	99.34 ± 1.2
AMX (12)	0.09 ± 0.0014***	125.67 ± 1.75**	0.98 ± 0.072*	0.76 ± 0.032***	99.13 ± 0.2
COT (12)	0.07 ± 0.0053 **	94 ± 0.93**	1.76 ± 0.032***	0.64 ± 0.047**	98.23 ± 0.79
NAL (10)	0.06 ± 0.001 *	109.17 ± 3.12**	0.99 ± 0.042*	0.53 ± 0.029**	98.56 ± 0.92
AMP (8)	0.06 ± 0.001*	328.80 ± 2.96***	1.53 ± 0.22***	0.79 ± 0.029***	98.76 ± 1.02
AZM (2)	0.02 ± 0.0014	24.40 ± 2.10	0.54 ± 0.022	0.24 ± 0.032	99.12 ± 0.79

[a] Pyocyanin production was expressed as OD_{520} after the incubation with 0.2N HCl. [b] Pyoverdine production was expressed as relative florescent units at 465nm (excitation λ=405nm) per mL of the culture. [c] Biofilm production was expressed as OD_{520} after incubating with crystal violet. [d] EPS production was expressed as OD_{490} after the 5% Phenol-H_2SO_4 (1:5 v/v) treatment. [e] Cell density was expressed as % growth per mL of the culture in comparison with the growth control. ns- non significant: * significant at p<0.05: **significant at p<0.01 and ***significant at p<0.001.

DISCUSSION

Catheter associated urinary tract infection is one of the most common nosocomial infections [2, 4, 5, 17], and its pathogenesis is related to the susceptibility of inert catheter material to microbial colonization and biofilm formation. Survival advantages conferred by biofilm community include resistance against-being swept away by shear forces, phagocytosis and anti-microbial agents. Due to these aspects it is difficult to completely eradicate the biofilm associated nosocomial infections, which causes unwanted clinical and economic outcomes [33, 34]. Currently no proven strategies are available for the prevention of CAUTI in chronically catheterized conditions [14].

Attenuation of the pathogens' virulence has been gaining interest as a new anti-infective approach. Depletion in pathogenicity will make the microbes incompatible to adapt and proliferate in host milieu hence readily cleared by the immune system [19]. For instance SIC levels of AZM found to be effective against cystic fibrosis (CF) by reducing the virulence factor expression through interfering with QS mechanism in *P. aeruginosa* and used as an effective method to prevent the complications [35]. Since *P. aeruginosa* is one of the major causative agents for CAUTI, and majority of the VFs was found to be QS controlled, attenuation of pathogenicity by the means of QSI has been found to be a potent plan of action against infections [25]. In the present study we have investigated the inhibitory effect of SIC levels of selected antibiotics upon the QS controlled virulence factor expression in test organisms that cause CAUTI. So that these antibiotics can be used as an improved biomaterial coating and / or as lock solution to prevent device associated infections.

The SIC of the selected antibiotics were assessed from the obtained MIC against the test organisms (Table 1). The test organisms used were; laboratory reference strain *P.aeruginosa* PAO1 and 2 clinical isolates of *Pseudomonas* sp. from CAUTI patients (PA-1 and PA-2). Choice of SIC as assay concentrations was also supported by the finding that anti-QS compounds permit unabated cell proliferation hence minimize the risk of emergence of resistance [36]. Among the clinical isolates, PA-2 exhibited similar MIC pattern with PAO1, while PA-1 displayed an elevated level in MIC than PAO1. Similar to the MIC profile, PA-2 had comparable virulence factor production with that of PAO1, while PA-2 evinced a raised virulence factor production (Table 2, 3 and 4).

Comparing the VFs produced by *P. aeruginosa* strains from UTI and CF patients, concluded that significant variation was observed in the expression pattern depending on the site of infection [37]. Features like enzymes, siderophores, toxins, EPS production and biofilm mode of growth help to evade antibiotic shock and host defense mechanisms. All these pathogenic aspects were under QS switch and vital for the induction of UTI as well [25]. Accordingly, the antibiotics were tested for their ability to inhibit the QS regulated production of the VFs in *P. aeruginosa* like pyocyanin, pyoverdine, biofilm formation and EPS formation [32]. Macrolide antibiotic AZM was used as a positive control and the anti-QS activity of the antibiotics were compared with that of the positive control.

Reduction in pyocyanin production has been largely used as an analysis in anti-QS research. Since the pyocyanin production is directly regulated by the QS circuit (*rhlR/rhlI*), reduction in production can be directly correlated with the reduction in the transcription levels [29, 30, 32]. In the present study, all the tested antibiotics at distinct concentrations reduced the pyocyanin production by the test organisms (Table 2, 3 and 4). Among the antibiotics, CXM reduced the test organisms' QS regulated pyocyanin production in a similar pattern with that of positive control. Other antibiotics also inhibited pyocyanin production; however the reduction rates were not proportionate with the positive control.

Pseudomonas quinolone signal (PQS) has been found to regulate many of the VFs in *P. aeruginosa* through activating *rhl* quorum sensing circuit. Production of siderophore pyoverdine was found to be coordinated by *rhl* QS system, influenced by the PQS concentration. Hence alterations in pyoverdine production have been used for the anti-QS investigations [31]. Though all the antibiotics, tested in the current study, showed reduction in pyoverdine production by the test organisms, CXM showed similar pattern with that of the positive control (Table 2, 3 and 4). Except AMX and AMP, NAL and COT significantly inhibited pyoverdine production by clinical isolate PA-1. Similarly in PA-2, except AMP, all the other antibiotics reduced pyoverdine production.

It has been extensively reported that both *las* and *rhl* systems enact in biofilm formation and maturation in *P. aeruginosa*. Hence, reduction in biofilm biomass has been recognized as a gold standard for the anti-QS property of test compounds [29, 31, 32]. The result of the biofilm bioassay of the present study indicated a reduction in the biofilm biomass by all the test organisms after the treatment with all the antibiotics (Table-2, 3 and 4). There was a significant reduction in biofilm formation by PAO1 treated with AMX in comparison with the positive control. Besides, CXM, COT and NAL showed similar inhibition pattern, while AMP exhibited the least inhibition. Among the clinical isolates, maximum reduction in biofilm formation by PA-1 followed the order CXM>NAL>AMX. In PA-2; CXM, AMX and NAL showed similar inhibition pattern while COT and AMP exhibited an incomparable reduction profile.

Quorum sensing controlled production of EPS has been found to be very important for the architectural integrity of biofilms. Hence reduction in EPS production is also examined to analyze the anti-QS property of test compounds [38]. In our study a significant reduction in EPS production by PAO1 was observed with CXM in comparison with the other test compounds. CXM exhibited utmost inhibition of EPS production in both the clinical isolates. All the other antibiotics had less comparable inhibition pattern with other test compounds (Table 2, 3 and 4).

It has been well documented that interfering with the QS mechanism does not inhibit growth. All the assays performed in the study were at sub-MIC levels, which had no effects on the growth of test organisms. The cell density analysis (Table 2, 3 and 4) at the tested concentrations allowed unabated cell growth and it can be concluded that in all the assays the reduction in the expression of VFs was not influenced by the reduction in the cell population [39].

Skindersoe *et al.,* (2008) analyzed the anti-QS property of 12 antibiotics against *P. aeruginosa* PAO1 and found 3 antibiotics, Azithromycin (AZM), ceftazidime (CFT) and ciprofloxacin (CPR) with potent activity at their sub-MIC levels. All the antibiotics tested in our study exhibited reduction in virulence factor production in all the test organisms; however, CXM, which is a cephalosporin antibiotic, like CPR [40] exhibited comparable outcome with the positive control.

Since the antibiotics used in the study were structurally different and enormous, the possibility of a high affinity competitive receptor site binding was of lesser feasibility. This hypothesis was supported by the *in silico* docking study results of antibiotics with the QS receptor sites, by Skindersoe *et al*., (2008) [40]. Elevated spacial penalties due to the improper fitting have been resulted in reduced affinity scores in docking studies. So it's been nearly confirmed that the underplaying mechanism for the anti-QS activity of the tested antibiotics will be other than a direct interaction with the QS receptor protein [40]. We speculate that the adaptive response of microbes towards stress conditions could be the mechanism for the observed anti-QS property of the tested antibiotics. Reduction in membrane permeability has been found to be a prime response adopted by microbes in response to stress conditions, which bring about decreased influx concentration of QS signal molecules, resulting in reduced expression levels of QS regulated genes [41, 42].

It has been reported that antibiotics display pleotroipic effects in clinical and environmental conditions, which are the practical demonstrations of the hormesis effect [41]. This concept was examined in our study by comparing the anti-QS property of the antibiotics with 2 clinical isolates from CAUTI patients (PA-1 and PA-2). The test compounds exhibited diverse inhibition pattern in all the tested QS regulated traits of clinical isolates, contrast to the laboratory reference strain. In other study, conducted by Wagner *et al*., (2005) compared the effects of AZM upon the expression of VFs by reference strains and clinical isolates from CF patients. CF clinical isolates exhibited a variable expression pattern in comparison to the reference strains. This variation could be due to the slow growth and limited exoproduct expression rate of clinical isolates in comparison to the reference strains [41]. But we speculate that, unlike in reference strains, clinical strains were capable to overcome the antibiotic stress by producing diverse exo-enzymes which could cleave antibiotics to small molecules. These small molecules could specifically bind to the QS receptors like conventional QSIs.

A significant variation in the effective dosage of the tested antibiotics was visible among all the test organisms. Even same antibiotics showed significant QSI activity against different test organisms at various concentrations. Similarly among the clinical isolates, alike antibiotics exhibited varied effects at diverse concentrations. This variation in the effective concentration can be due to the change in

the transcription levels of the various characters. The contrast in the observations could be due to the slow growth rate and the limited virulence factor production rate of clinical isolates in the *in vitro* conditions. The lack of these responses might be due to the different intracellular concentrations of the test compounds with corresponding hermetic effects as well [43].

All the antibiotics tested in the study exhibit diverse mechanisms of antimicrobial action and are structurally very different. Thus it is interesting that all the antibiotics exhibited reduction in QS regulated characters at varied degrees and at diverse concentrations indicating that the observed QSI action has less correlation with their anti-microbial mechanism of action [43, 41]. A detailed reverse transcription PCR and DNA-microarray analysis will provide a clear cut idea about the mechanism as well as the cluster of genes which are affected by the hermetic action of the test compounds [40, 41]. Since the present study compares the effect of anti-QS activity of the antibiotics upon QS regulated behaviors of both PAO1 and CAUTI isolates, further detailed study in this aspect warranted to design a QS dependent therapy against nosocomial infections like CAUTI.

CONCLUSION

To the best of our knowledge, this is the first report demonstrating the anti-QS property of CXM against PAO1 and 2 other *Pseudomonas* sp. clinical isolates from CAUTI patients. QS Inhibitory property of the test compound indicates its propitious anti-infective drug against CAUTI. Since the chances of resistance development is unlikely, since at SIC levels the test compounds pose no or little selective pressure. A further comprehensive study using CXM coated or wash solutions against chronically catheterized conditions will envisage the actual clinical impact of the observations that made in the study.

ACKNOWLEDGEMENT

The authors express their gratitude to Jeniffer James, Deenu Sudesh and Romey A Mathew, who helped in generating the preliminary data for the study. Authors are also thankful to VIT University, for the financial assistance to conduct this study.

REFERENCES

1. Jahn P, Preuss M, Kernig A, Seifert HA and Langer G. Types of indwelling urinary catheters for long-term bladder drainage in adults. Cochrane Database Syst Rev. 2007; 18(3): CD004997.
2. Johnson JR, Delavari P and Azar M. Activities of a Nitrofurazone-Containing Urinary Catheter and a Silver Hydrogel Catheter against Multidrug-Resistant Bacteria Characteristic of Catheter-Associated Urinary Tract Infection. Antimicrob Agents Chemother**.** 1999; 43(12): 2990 - 5.
3. Falaqas ME and Makris GC. Probiotic bacteria and biosurfactants for nosocomial infection control: a hypothesis. J Hosp Infect. 2009; 71(4): 301 - 6.
4. Nicolle L. The chronic indwelling catheter and urinary infection in long-term-care facility residents. Infect Control Hosp Epidemiol. 2001; 22: 316 - 21.
5. Warren, J. Nosocomial urinary tract infections. In: Mandell G, Bennett J, Dolin R, editors. Principles and Practice of Infectious Diseases. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 3028.
6. Warren J. Catheter-associated urinary tract infections. Infect Dis Clin North Am. 1997; 11: 609 - 22.
7. Tambyah P and Maki D. Catheter-associated urinary tract infection is rarely symptomatic. Arch Intern Med. 2000; 160: 678 - 82.
8. Gillespie W, Jones J, Teasdale C, Simpson R, Nashef L and Speller D. Does the addition of disinfectant to urine drainage bags prevent infection in catheterized patients? Lancet. 1983; 1: 1037 - 9.
9. Maizels M and Schaeffer AJ. Decreased incidence of bacteriuria associated with periodic instillations of hydrogen peroxide into the urethral catheter drainage bag. J Urol. 1980; 123: 841 - 5.
10. Pearman JW. The value of kanamycin-colistin bladder instillations in reducing bacteriuria during intermittent catheterisation of patients with acute spinal cord injury. Br J Urol. 1979; 51: 367 - 74.
11. Schaeffer AJ, Story KO and Johnson SM. Effect of silver oxide/trichloroisocyanuric acid antimicrobial urinary drainage system on catheter-associated bacteriuria. J Urol. 1988; 139: 69 - 73.
12. Saint S, Elmore JG, Sullivan SD, Emerson SS and Koepsell TD. The efficacy of silver alloy-coated urinary catheters in preventing urinary tract infection: a meta-analysis. Am J Med. 1998; 105: 236 - 41.
13. Trauntner BW and Darouiche RO. Role of biofilm in catheter-associated urinary tract infection. Am J Infect Control. 2004; 32(3): 177–83.
14. Brosnahan J, Jull A and Tracy C. Types of urethral catheters for management of short-term voiding problems in hospitalised adults. Cochrane Database Syst Rev. 2004; (1): CD004013.
15. Maki DG and Tambyah PA. Engineering out the risk for infection with urinary catheters. Emerg Infect Dis. 2001; 7(2): 342 - 7.
16. Warren JW, Platt R, Thomas RJ, Rosner B and Kass EH. Antibiotic irrigation and catheter-associated urinary-tract infections. N Engl J Med. 1978; 299(11): 570 - 3.
17. Jarvis WR and Martone WJ. Predominant pathogens in hospital infections. J Antimicrob Chemother**.** 1992; 29: 19 - 24.
18. Denstedt J, Wollin T and Reid G. Biomaterials used in urology: current issues of biocompatibility, infection, and encrustation. J Endourol. 1998; 12: 493 - 500.
19. Clatworthy AE, Pierson E and Hung DT. Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol. 2007; 3(9): 541 - 8.
20. Miller MB and Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol**.** 2001; 55: 165 - 199.
21. Ganin H, Tang X and Meijler MM. Inhibition of *Pseudomonas aeruginosa* quorum sensing by AI-2 analogs. Bioorg Med Chem Lett. 2009; 19(14): 3941 - 4.
22. VanBogelen RA and Neidhardt FC. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc Natl Acad Sci USA**.** 1990; 87: 5589 - 5593.
23. Nalca Y, Jansch L, Bredenbruch F, Geffers R and Buer J, Haussler S. Quorum-Sensing Antagonistic Activities of Azithromycin in *Pseudomonas aeruginosa* PAO1: a Global Approach. Antimicrob Agents Chemother. 2006; 50(5): 1680 - 8.
24. Skindersoe ME, Alhede M, Phipps R, Yang L, Jensen PO, Rasmussen TB, *et al*. Effects of antibiotics on quorum sensing in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2008; 52(10): 3648 - 63.
25. Mittal R, Aggarwal S, Sharma S, Chhibber S and Harjai K. Urinary tract infections caused by Pseudomonas aeruginosa: a minireview. J Infect Public Health. 2009; 2(3): 101 - 11.
26. Gambello MJ and Iglewski BH. Cloning and characterization of the *Pseudomonas aeruginosa* lasR gene, a tran scriptional activator of elastase expression. J Bacteriol. 1991; 173: 3000 - 9.
27. Ochsner UA and Reiser J. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA. 1995; 92: 6424 - 8.
28. Witker M, Cockerill F, Craig W, Dudley M, Eliopoulos G, Hecht D et al. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard-seventh edition. Clinical and Laboratory Standards Institute document M7-A7. USA: Clinical and Laboratory Standards Institute. 2006.
29. Syed K, Bhagavathi M and Sivamaruthi S. Quorum Sensing Inhibition in *Pseudomonas aeruginosa* PAO1 by Antagonistic Compound Phenylacetic Acid. Curr Microbiol. 2012; 65(5): 475 - 80.
30. Cox CD and Adams P. Siderophore Activity of Pyoverdine for *Pseudomonas aeruginosa*. Infect Immun. 1985; 48(1):130 - 8.
31. Adonizio A, Kong KF and Mathee K. Inhibition of Quorum Sensing-Controlled Virulence Factor Production in *Pseudomonas aeruginosa* by South Florida Plant Extracts. Antimicrob Agents Chemother. 2008; 52(1): 198 - 203.
32. Packiavathy IA, Priya S, Pandian SK and Ravi AV. Inhibition of biofilm development of uropathogens by curcumin-An anti-quorum sensing agent from *Curcuma longa*. Food Chem. 2012; 148: 453 - 60.
33. Costerton J, Geesey G and Cheng K. How bacteria stick. Sci Am. 1978; 238: 86 - 95.

34. Costerton J, Lewandowski S, Caldwell D, Korber D, Lappin-Scott H. Microbial biofilms. Annu Rev Microbiol. 1995; 49: 711 - 45.
35. Nalca Y, Jansch L, Bredenbruch F, Geffers R, Buer J and Haussler S. Quorum-Sensing Antagonistic Activities of Azithromycin in *Pseudomonas aeruginosa* PAO1: a Global Approach. Antimicrob Agents Chemother. 2006; 50(5): 1680 - 8.
36. Defoirdt T, Boon N and Bossier P. Can bacteria evolve resistance to quorum sensing disruption?. PLoS Pathog. 2010; 6(7): e1000989.
37. Ciragil P and Söyletir G. Alginate, elastase and alkaline protease production of *Pseudomonas aeruginosa* strains isolated from various body sites. Mikrobiyol Bul**.** 2004; 38(4): 341 - 7.
38. Ohman DE, Cryz SJ and Iglewski BH. Isolation and Characterization of a *Pseudomonas aeruginosa* PAO Mutant That Produces Altered Elastase. J Bacteriol. 1980; 142(3): 836 - 42.
39. Clec'h C, Schwebel C, Français A, Toledano D, Fosse J-P, Garrouste-Orgeas M, *et al*., Does catheter-associated urinary tract infection increase mortality in critically ill patients? Infect Control Hosp Epidemiol. 2007; 28(12): 1367 - 73.
40. Skindersoe ME, Alhede M, Phipps R, Yang L, Jensen PO, Rasmussen TB, et al. Effects of antibiotics on quorum sensing in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2008; 52(10): 3648 - 63.
41. Wagner T, Soong G, Sokol S and Saiman L. Effects of Azithromycin on Clinical Isolates of *Pseudomonas aeruginosa* From Cystic Fibrosis Patients. Chest. 2005; 128(2): 912 - 9.
42. Ciofu O, Giwercman B, Pedersen SS and Høiby N. Development of antibiotic resistance in *Pseudomonas aeruginosa* during two decades of anti-pseudomonal treatment at the Danish CF Center. APMIS. 1994; 102(9): 674 - 80.
43. Davies J, Spiegelman GB and Yim G. The world of sub-inhibitory antibiotic concentrations. Curr Opin Microbiol. 2006; 9(5): 445 - 53.