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A proposed approach to correlate antibiotic and biocide resistance in staphylococcal isolates based on Minimum Inhibitoy Concentration (MIC) data analyses

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ABSTRACT

The study aimed to compare the sensitivity of *Staphylococcus* isolates from hospital and non hospital sources to some commonly used antibiotics and biocides, and to investigate whether resistance to either types of antimicrobials are correlated. The minimum inhibitory concentrations (MICs) of the antibiotics and biocides were determined for clinical (47), hospital environment (64) and non hospital (33) isolates using agar dilution method. Arithmetic progression in biocides concentration was used instead of the geometric progression for antibiotics. Pearson's correlation coefficients (r) between MIC-MIC, Log MIC-Log MIC, and MIC-Log MIC for individual biocides and antibiotics, respectively, were compared. Also, the frequencies of antibiotic resistance among biocide-susceptible and biocide-resistant isolates were calculated. The antibiotics included: ampicillin, penicillin G, oxacillin, cefepime, streptomycin, tetracycline, gentamicin, azithromycin, ciprofloxacin, vancomycin and chloramphenicol, while biocides included chlorocresol, benzalkonium chloride, cetrimide, chlorhexidine, phenyl mercuric nitrate, povidone-iodine and ethidium bromide. Hospital isolates showed higher resistance rates to antibiotics and biocides and more predominance of methicillin resistance compared to non-hospital isolates. The most likely correlation between antibiotic and biocide resistance was best expressed by comparing log MIC of antibiotic with MIC of biocide. For hospital isolates, positive correlations were found between increased resistance to most of tested antibiotics (except for vancomycin and occasionally ciprofloxacin and tetracycline) and reduced susceptibility to biocides, except for chlorhexidine and povidone-iodine. For non hospital isolates, only resistance to benzalkonium chloride and cetrimide correlated with resistance to most of the tested antibiotics. Except for chlorhexidine and povidone-Iodine higher resistance rates to antibiotics were found among biocide resistant isolates.

Keywords: co-resistance; antibiotics; biocides; staphylococcus, MRSA; hospital; non-hospital

INTRODUCTION

Microbial resistance to antibiotics is an increasing problem. Some evidences suggest that exposure to biocides (antiseptics and disinfectants) used in various setting may contribute to the increased occurrence of antibiotic resistance (Russell et al., 1998 &1999; Fraise 2002**;** Walsh et al., 2003; Sheldon et al.,2005 ; Weber and Rutala, 2006; Carson et al., 2008).

Antibiotic and biocide antibacterial actions show many similarities despite some differences in terms of target, killing behaviour, clinical aspects (Poole 2007) and definition of resistance (McDonnell and Russell, 1999; Russell, 2003). Some mechanisms of resistance, like efflux pumps, are common to both biocides and antibiotics and have been shown to act on a range of chemically dissimilar compounds (Levy, 2002; Maillard, 2007; Poole, 2007).

Although the role of biocide use on development of antibiotic resistance is believed to be over emphasized (Jones, 1999), research on the impact of biocide use on spread of antimicrobial resistance is urged (the Scientific Committee on Emerging and Newly Identified Health Risks, SCENIHR,

2009). However, there are no standardized testing protocols that measure both biocide and antibiotic resistance in bacteria (SCENIHR, 2009). Although many reports of bacterial resistance to biocides are based on MIC data, using MIC to measure bacterial resistance is arguable (Russell and McDonnell 2000, Russell et al., 1998). Unlike antibiotic, where resistance is manifested by very sharp change in MIC due to lack of or reduced susceptibility to the specific target, the biocide can affect multiple targets in microbial cell and loss of one of these targets leaves others still responsive (McDonnell and Russell, 1999). This raises the question whether the two fold serial dilution protocol adopted for determination of MIC for antibiotics is a sensitive enough to detect the slight changes in susceptibility expected with biocides. There is a need for the development of standard protocols for the quantitative assessment of biocide induced resistance and cross resistance (SCENIHR, 2009).

S. aureus is a popular nosocomial pathogen that presents a therapeutic problem due to its ability to rapidly acquire resistance to frequently used drugs. The objective of the present study was to: first, verify whether MICs can be employed to correlate resistance to biocides and antibiotics in *Staphylococcus* species; and secondly, to find whether this possible correlation could vary according to isolates' background by comparing MICs for isolates from various sources with the likelihood of exposure to biocides and/or antibiotics.

MATERIALS and METHODS:

Bacterial isolates

The study involved 140 staphylococcal isolates, comprising 47 clinical hospital isolates, 64 hospital environmental isolates from Zagazig University Hospitals and 33 non hospital environmental isolates collected during the period between May 2005 and June 2006. The isolates were identified according to Koneman et al (1997).

Antimicrobials and culture media

Pharmaceutical grades of antibiotics and biocides were obtained from the local pharmaceutical companies. Oxacillin , vancomycin, chlorhexidine HCl, benzalkonium chloride, cetrimide, phenyl mercuric nitrate, ethidium bromide and chlorocresol were obtained from Sigma Chemical Company, St. Louis, Mo, USA. Culture media and oxacillin discs were obtained form from Oxoid, Hampshire, England.

Detection of methicillin resistance

Detection of methicillin resistance was carried out using oxacillin discs (1 μg) according to NCCLS (1993).

Determination of minimum inhibitory concentration (MIC)

The MICs of antimicrobial agents were determined using agar dilution method according to NCCLS (1997). For biocides, increasing concentrations with constant increments (arithmetic progression) were made in Mueller-Hinton agar instead of two-fold serial dilution. The isolates were categorized as antibiotic resistant or sensitive according to NCCL breakpoints. Resistance breakpoints to chlorocresol, benzalkonium chloride, cetrimide, chlorhexidine, phenyl mercuric nitrate, ethidium bromide and povidone-Iodine was assumed by MIC values equal to or greater than 250 μg ml^{-1} , 5.0 μg ml^{-1} , 8.0 μg ml^{-1} , 2.0 μg ml^{-1} , 0.2 μg ml^{-1} , 10.0 μg ml^{-1} , and 2500 μg ml^{-1}, respectively.

Statistical Analysis

To investigate the degree of cross-resistance between two antimicrobials, Pearson's correlation coefficients (r), which give a measure of the strength of any linear relationship between MIC values (or their log_{10} derivatives) of the two antimicrobials,

were calculated using Prism software (GraphPad software, Inc. La Jolla, CA, USA version 5.01) at the same two tailed P <0.05. For an exact relationship, the correlation is 1 or -1, depending on the relationship and if there is no linear relationship, the correlation tends to zero. MIC data were transformed into $\log_{10}$ MIC and either MIC-MIC, log MIC-MIC, or log MIC-log MIC combinations for antibiotics and biocides, respectively were used and data were compared for magnitude of the coefficient and discrepancies between them in terms of significance and non significance.

RESULTS:

Detection of methicillin resistance among *Staphylococcus* isolates

Higher percentages of methicillin resistant isolates were found among clinical (31.9%), and hospital environment isolates (28.1%) compared to non hospital environmental isolates (9.1%).

Resistance of *Staphylococcus* isolates to antimicrobial agents

Relatively higher resistance rates to individual antibiotics and biocides were found among isolates from hospital compared to non hospital isolates (Table 1).

Table 1. Percentage *Staphylococcus* isolates resistant antibiotics and biocides*

Antimicrobial agents	Resistance breakpoint concentration (µg ml^{-1})	Percent of resistant isolates among		
		Clinical isolates n=47	hospital environment isolates n=64	non-hospital environment isolates n=33
penicillin G	0. 25	70.2	53.1	36.4
Ampicillin	0.25	70.2	46.9	36.4
Oxacillin	4	31.9	28.1	9.1
Vancomycin	2	0	0	0
Cefepime	64	29.8	15.6	12.1
Streptomycin	16	31.9	42.2	36.4
Tetracycline	16	29.8	35.9	21.2
Gentamicin	16	17	9.4	3.0
Azithromycin	8	36.2	42.2	9.1
Ciprofloxacin	4	19.1	23.4	15.2
Chloramphenicol	32	12.8	4.7	0
Chlorocresol	250	27.7	21.9	12.1
Benzalkonium chloride	4	59.6	60.9	48.5
Cetrimide	8	19.1	34.4	21.2
Phenyl mercuric nitrate	0.2	51.1	59.4	39.4
Ethidium bromide	10	12.2	25	0
Povidone-Iodine	2500	70.2	79.7	54.5

*** Biocide resistance calculated based on the suggested breakpoint concentration**

Comparison of susceptibility of isolates to biocides and antibiotics

Pearson's correlation coefficient (r) between MIC-MIC, MIC-log MIC, and log MIC-log MIC were calculated for individual groups and total isolates. Pearson's coefficients (r) for MIC values and their significance are shown in tables (2-5). In case of discrepancy in the interpretation of significance of correlation between MIC-MIC comparison and each of log MIC-MIC or log MIC-log MIC, it was indicated as superscript.

Table 2. Pearson's correlation coefficient between antibiotic and biocide MIC for clinical isolates and its significance of correlation

Antibiotic/biocide	CC	BKC	CET	CHX	PMN	PI	EB
Penicillin G	0.64***	0.47***	0.59***	-0.04NS	0.21NS[a]	-0.45**[d]	0.43NS[a]
Ampicillin	0.62***	-0.34**	0.39***	0.13NS	0.23NS[a]	-0.37*[d]	0.34NS[a]
Oxacillin	0.53***	0.47***	0.54***	0.17NS	0.26NS	-0.33*[cd]	0.26NS[ab]
Cefepime	0.64***	0.11**	0.34*	0.02NS	0.35*	-0.46***[d]	0.27NS[ab]
Vancomycin	-0.29 NS	0.24NS	-0.19NS	0.44NS[ab]	-0.09NS	0.29*[c]	-0.14NS
Streptomycin	0.65***	0.47***	0.50***	-0.17NS	0.25NS	-0.55***[cd]	0.37*
Gentamicin	0.45**	0.53NS[b]	-0.057NS	-0.05NS	0.14NS	-0.35*[cd]	-0.08***[cd]
Tetracycline	0.39**[d]	0.06NS	0.022NS	-0.04NS	0.35NS	-0.35*[cd]	0.09NS
Azithromycin	0.32*	0.09NS[ab]	0.17NS[ab]	0.09NS	0.17NS	-0.24NS	0.21NS[ab]
Chloramphenicol	0.48***	0.516NS	-0.08NS	-0.07NS	0.24NS	-0.41**[cd]	-0.12*[cd]
Ciprofloxacin	0.13NS[ab]	-0.34***	0.59***	-0.03NS	-0.10NS	-0.11NS	0.85NS[ab]

[*] significant correlation(p between 0.01 and 0.05)
[**] very significant correlation (p is less than 0.01)
[***] extremely significant correlation (p is less than 0.001)
[a] significant with antibiotic log MIC versus biocide MIC
[b] significant with antibiotic log MIC versus biocide log MIC
[c] non-significant with antibiotic log MIC versus biocide MIC
[d] non-significant with antibiotic log MIC versus biocide log MIC

Table 3. Pearson's correlation coefficient between antibiotic and biocide MIC for Hospital environmental isolates and its significance of correlation

Antibiotic/biocide	CC	BKC	CET	CHX	PMN	PI	EB
Penicillin G	0.41***	0.54***	0.64***	-0.10NS	0.26* [c]	0.13NS	0.50***
Ampicillin	0.38**	0.46***	0.55***	-0.06NS	0.23NS	0.09NS	0.44***
Oxacillin	0.42***	0.57***	0.66***	0.031NS	0.33**	0.08NS	0.56***
Cefepime	0.446***	0.38**	0.48***	-0.03NS	0.33**	-0.03NS	0.36**
Vancomycin	0.27*[cd]	0.22NS	0.33**	-0.15NS	0.18NS	0.15NS	0.19NS[b]
Streptomycin	0.57***	0.63***	0.64***	-0.06NS	0.34**	0.08NS	0.57***
Gentamicin	0.39**	0.32**	0.13NS[ab]	0.01NS	0.19NS	-0.18NS	0.33**
Tetracycline	0.36NS	0.17NS[ab]	-0.04NS	-0.04NS	0.15NS	-0.17NS	0.02NS
Azithromycin	0.27*	0.32**	0.30*	0.12NS	0.17NS[b]	-0.22NS	0.31*
Chloramphenicol	0.35**	0.39**	0.22NS[ab]	0.14NS	0.18NS	-0.17NS	0.51***
Ciprofloxacin	0.22NS[a]	0.19NS[ab]	0.33**	-0.06NS	0.27*	0.09NS	0.22NS[ab]

[*] significant correlation(p between 0.01 and 0.05)
[**] very significant correlation (p is less than 0.01)
[***] extremely significant correlation (p is less than 0.001)
[a] significant with antibiotic log MIC versus biocide MIC
[b] significant with antibiotic log MIC versus biocide log MIC
[c] non-significant with antibiotic log MIC versus biocide MIC
[d] non-significant with antibiotic log MIC versus biocide log MIC

Table 4. Pearson's correlation coefficient between antibiotic and biocide MIC for environmental isolates and its significance of correlation

Antibiotic/biocide	CC	BKC	CET	CHX	PMN	PI	EB
Penicillin G	0.01NS	0.35*[cd]	0.86***	-0.16NS	0.12NS	0.02NS	0.23NS
Ampicillin	0.12NS	0.32NS	0.82***	-0.21NS	0.09NS	0.04NS	0.24NS
Oxacillin	0.34NS	0.61***	0.97***	-0.14NS	0.29NS[ab]	-0.09NS[ab]	0.16NS
Cefepime	0.28NS	0.52** [d]	0.97***	-0.14NS	0.25NS	-0.11NS	0.16NS
Vancomycin	0.17NS	-0.12NS	-0.04NS	0.12NS	-0.29NS	0.09NS	0.04NS
Streptomycin	0.08NS	0.25NS	0.45**	-0.24NS	0.02NS	-0.21NS	0.35*
Gentamicin	0.27NS	0.43* [cd]	0.91***	-0.09NS	0.20NS	-0.21NS	0.12NS
Tetracycline	-0.23NS	0.34NS	0.09NS	-0.32NS	0.19NS	0.04NS	0.11NS
Azithromycin	-0.19NS	0.39*	0..017NS	-0.14NS	0.31NS	-0.08NS	-0.11NS
Chloramphenicol	-0.23NS	0.51**	0.39NS[b]	-0.47**[cd]	0.27NS	-0.11NS	0.07NS
Ciprofloxacin	-0.27NS	0.35*	0.12NS[b]	-0.29NS	0.16NS	0.06NS	0.15NS

[*] significant correlation(p between 0.01 and 0.05)
[**] very significant correlation (p is less than 0.01)
[***] extremely significant correlation (p is less than 0.001)
[a] significant with antibiotic log MIC versus biocide MIC
[b] significant with antibiotic log MIC versus biocide log MIC
[c] non-significant with antibiotic log MIC versus biocide MIC
[d] non-significant with antibiotic log MIC versus biocide log MIC

Table 5. Pearson's correlation coefficient between antibiotic and biocide MIC for combined isolates and its significance of correlation

Antibiotic/biocide	CC	BKC	CET	CHX	PMN	PVI	EB
Penicillin G	0.44***	0.52***	0.64***	-0.05NS	0.22**	-0.08NS	0.46***
Ampicillin	0.44***	0.41***	0.46***	0.08NS	0.21*	-0.11NS	0.36***
Oxacillin	0.41***	0.52***	0.63***	0.12NS	0.29***	-0.10NS	0.39***
Cefepime	0.46***	0.37***	0.42***	0.03NS	0.31***	-0.18*	0.32***
Vancomycin	0.00NS	-0.04NS	0.07NS	0.15NS	-0.02NS	0.19*	0.06NS
Streptomycin	0.51***	0.50***	0.555***	-0.11NS	0.23**	-0.21*	0.43***
Gentamicin	0.31***	0.11NS	0.00NS	-0.00NS	0.11NS[ab]	-0.21*	0.03NS
Tetracycline	0.19*[cd]	0.17*	0.01NS[b]	-0.04NS	0.14NS[b]	-0.19*	0.06NS
Azithromycin	0.23**	0.28***	0.25**	0.12NS	0.19*	-0.15NS	0.30***
chloramphenicol	0.344**	0.19*	0.06NS	0.03NS	0.19*	-0.24**[cd]	0.17*[cd]
Ciprofloxacin	0.14NS[ab]	0.33***	0.42***	-0.03NS	0.12NS[ab]	0.02NS	0.45***

[*] significant correlation(p between 0.01 and 0.05)
[**] very significant correlation (p is less than 0.01)
[***] extremely significant correlation (p is less than 0.001)
[a] significant with antibiotic log MIC versus biocide MIC
[b] significant with antibiotic log MIC versus biocide log MIC
[c] non-significant with antibiotic log MIC versus biocide MIC
[d] non-significant with antibiotic log MIC versus biocide log MIC

Further analyses for MIC data based on comparing the percentages of antibiotic resistant isolates among biocide-susceptible and biocide-resistant ones are presented in tables (6-12).

Table 6. The percentage of antibiotic resistant isolates among chlorhexidine susceptible and resistant isolates.

Type of isolates	Clinical (n=47)		Hospital env (n=64)		Non hosp env (n=33)		Total (n= 144)	
antibiotic	S =37	R =10	S =53	R =11	S =33	R= 0	S=123 (85.4%)	R= 21
Penicillin	86.5	10	62.3	9.1	12.1	0.0	62.6	9.5
Ampicillin	86.5	10	54.7	9.1	36.4	0.0	59.3	9.5
Oxacillin	37.8	10	32.1	9.1	9.1	0.0	27.6	9.5
Cefepime	35.1	10	17.0	9.1	12.1	0.0	21.1	9.5
Streptomycin	40.5	0.0	43.4	36.4	36.4	0.0	40.6	19.0
Tetracycline	29.7	30	43.4	0.0	21.2	0.0	27.6	14.3
Gentamicin	21.6	0.0	11.3	0.0	3.0	0.0	12.2	4.8
Azithromycin	40.5	20	43.4	36.4	9.1	0.0	33.3	28.6
Ciprofloxacin	24.3	0.0	26.4	9.1	15.1	0.0	22.8	4.8
Chloramphenicol	16.2	0.0	3.8	9.1	0	0.0	6.5	4.8

Table 7. The percentage of antibiotic resistant isolates among Chlorocresol susceptible and resistant isolates.

Type of isolates	Clinical		Hospital env		Non hosp env		Total	
Antibiotic	S=34	R =13	S=50	R =14	S =29	R= 4	S =113 (78.47%)	R =31
Penicillin	61.8	92.3	48	71.4	34.5	50	48.7	74
Ampicillin	61.8	92.3	40	71.4	34.5	50	45.1	74
Oxacillin	8.8	92.3	16	71.4	6.9	25	11.5	71
Cefepime	5.9	92.3	6	50	10.3	25	7.1	64.5
Streptomycin	11.8	84.6	26	100	34.5	50	(23.9	87.1
Tetracycline	20.6	53.8	36	35.7	20.7	25	27.4	41.9
Gentamicin	0.0	61.5	2	35.7	0.0	25	0.9	45.2
Azithromycin	14.7	92.3	34	71.4	6.9	25	(21.2	71
Ciprofloxacin	2.9	61.5	14	57.1	17.2	0.0	11.5	51.6
Chloramphenicol	0.0	46.2	0.0	21.4	0.0	0.0	0.0	29

Table 8. The percentage of antibiotic resistant isolates among Phenyl mercuric nitrate susceptible and resistant isolates.

Type of isolates	Clinical		Hospital env		Non hosp env		Total	
Antibiotic	S =23	R =24	S =26	R =38	S=20	R= 13	S= 69 (47.9%)	R= 75
Penicillin	60.9	79.2	46.2	57.9	35	38.5	47.8	61.3
Ampicillin	60.9	79.2	38.5	52.6	35	38.5	44.9	58.7
Oxacillin	17.4	45.8	15.4	36.8	0.0	23.1	11.6	37.3
Cefepime	13	45.8	0.0	26.3	5	23.1	5.8	32
Streptomycin	26.1	37.5	26.9	52.6	35	38.5	30	45.3
Tetracycline	30.7	29.2	30.8	39.5	15	30.8	26.1	34.7
Gentamicin	8.7	25	3.8	13.2	0.0	7.7	4.3	16
Azithromycin	21.7	50	26.9	52.6	0.0	23.1	17.4	46.7
Ciprofloxacin	8.7	29.2	3.8	36.8	10	23.1	6.7	32
Chloramphenicol	4.3	20.8	0.0	7.9	0.0	0.0	1.44	10.7

Table 9. The percentage of antibiotic resistant isolates among benzalkonium chloride susceptible and resistant isolates.

Type of isolates	Clinical isolates		Hospital env		Non hosp env		Total	
Antibiotic	S = 19	R =28	S= 25	R= 39	S =17	R= 16	S =61 (42.4%)	R =83
Penicillin G	31.6	96.4	48	56.4	41.2	31.2	41	65.1
Ampicillin	31.6	96.4	36	53.8	41.2	31.2	36.1	63.8
Oxacillin	5.3	50	8	41	0.0	18.7	4.9	39.7
Cefepime	0.0	50	0.0	25.6	5.9	18.7	1.6	32.5
Streptomycin	15.8	42.9	12	61.5	29.4	43.7	18	51.8
Tetracycline	31.6	28.6	24	43.6	17.6	25	24.6	34.9
Gentamicin	0.0	28.6	0.0	15.4	0.0	6.2	0.0	18.1
Azithromycin	21.1	46.4	28	51.3	0.0	8.7	18	43.4
Ciprofloxacin	0.0	32.1	16	28.2	11.8	18.7	9.8	27.7
Chloramphenicol	0.0	21.4	0.0	7.7	0.0	0.0	0.0	10.8

Table 10 . The percentage of antibiotic resistant isolates among cetrimide susceptible and resistant isolates.

Type of isolates	Clinical isolates		Hospital env		Non hosp env		Total	
Antibiotic	S = 38	R = 9	S =42	R = 22	S =26	R= 7	S =106 (73.6%)	R= 38
Penicillin	32.5	88.9	45.2	68.2	26.9	71.4	48.1	73.7
Ampicillin	32.5	88.9	35.7	68.2	26.9	71.4	44.3	73.7
Oxacillin	18.4	88.9	16.7	50	0.0	42.9	13.2	57.9
Cefepime	15.8	88.9	7.1	31.8	3.8	42.9	9.4	47.4
Streptomycin	18.4	88.9	35.7	54.5	19.2	100	25.5	71
Tetracycline	28.9	33.3	38.1	31.8	11.5	57.1	28.3	36.8
Gentamicin	13.2	33.3	4.8	18.2	0.0	14.3	6.6	21
Azithromycin	26.3	77.8	23.8	77.3	3.8	28.6	19.8	68.4
Ciprofloxacin	13.2	44.4	14.3	40.9	3.8	57.1	11.3	44.7
Chloramphenicol	10.5	22.2	2.4	9.1	0.0	0.0	4.7	7.9

Table 11 . The percentage of antibiotic resistant isolates among ethidium bromide susceptible and resistant isolates.

Type of isolates	Clinical isolates		Hospital env		Non hosp env		Total	
antibiotic	S =41	R =6	S = 48	R = 16	S =33	R (0)	S= 122 (84%)	R= 22
Penicillin	68.3	83.3	45.8	75	36.4	0.0	50.8	77.3
Ampicillin	68.3	83.3	37.5	75	36.4	0.0	47.5	77.3
Oxacillin	24.4	83.3	18.8	56.3	9.1	0.0	18	63.6
Cefepime	22	83.3	6.3	43.8	12.1	0.0	13.9	54.5
Streptomycin	22	100	33.3	68.8	36.4	0.0	30.3	77.3
Tetracycline	26.8	50	33.3	43.8	21.2	0.0	27.9	45.4
Gentamicin	14.6	33.3	6.3	18.8	3	0.0	8.2	22.7
Azithromycin	29.3	83.3	33.3	68.8	9.1	0.0	25.4	72.7
Ciprofloxacin	12.2	66.7	14.6	50	15.1	0.0	13.9	54.5
Chloramphenicol	14.6	0.0	2.1	12.5	0.0	0.0	5.7	9.1

Table 12 . The percentage of antibiotic resistant isolates among povidone-iodine susceptible and resistant isolates.

Type of isolates	Clinical isolates		Hospital env		Non hosp env		Total	
antibiotic	S =14	R =33	S = 12	R = 51	S =15	R=18	S= 41	R= 102 (71.5)
Penicillin	57.1	9.1	25	16.9	53.3	16.7	46.3	14.7
Ampicillin	57.1	15.2	25	16.9	53.3	27.8	46.3	18.6
Oxacillin	64.3	18.2	50	22.6	60	33.3	58.5	23.5
Cefepime	57.1	18.2	25	11.3	53.3	33.3	46.3	17.6
Streptomycin	57.1	21.2	50	39.6	53.3	38.9	53.7	34.3
Tetracycline	42.9	21.2	33.3	32	40	38.9	39	30.3
Gentamicin	50	3	16.7	7.5	46.7	5.6	39	5.8
Azithromycin	85.7	15.2	75	33.9	80	27.8	80.5	27.4
Ciprofloxacin	35.7	12.1	33.3	20.7	33.3	22.2	34.1	18.6
Chloramphenicol	35.7	3	8.3	1.8	33.3	5.6	26.8	2.9

Table 13. Comparison of the number of discrepancies in interpretation of significance and non significance for correlation between antibiotic and biocide resistance using MIC-MIC, logMIC-MIC, and log MIC-log MIC

Isolate type	Comparison of					
	MIC-MIC		Log MIC-MIC		Log MIC-log MIC	
	NS odds	S odds	NS odds	S odds	NS odds	S odds
Clinical isolates	5	-	-	2	3	1
Hospital env isolates	5	1	1	1	-	2
Environmental isolates	1	2	-	-	-	1
Combined isolates	8	4	-	1	-	4

DISCUSSION:

Much concern has been raised on the role of widespread use of biocides in various fields including healthcare setting on the development of antibiotic (emergence or selection) resistance among microbes (Russell et al., 1999; Levy 2002; Sheldon, 2005; Weber and Rutala, 2006). The present study aimed to investigate the antibiotic and biocide resistance profiles for staphylococcal isolates from hospital and non hospital sources and to find out any correlation (or cross resistance) between resistances to either agent.

The study focused on *Staphylococcus* species, as one of the most common pathogens in clinical setting with reputation of rapidly developing resistance to most chemotherapeutic antimicrobial agents (Johnson et al., 2003; Huang et al., 2007). The approach adopted in the present study was to recover staphylococcal isolates from various sources with likelihood of exposure to antibiotics, biocides, or both, if any, and to compare their susceptibility to selected group of antibiotics and biocides.

In general, the study clearly demonstrated higher methicillin and other antibiotic resistance rates among isolates from clinical and hospital environmental settings compared to the non hospital isolates. This was quite evident for the β-lactam antibiotics, azithromycin, and gentamicin, but not with streptomycin, tetracycline, and ciprofloxacin, probably reflecting the infrequent use of the first two and the widespread use of the latter.
The relatively higher rates in resistance to

antibiotics and biocides among hospital isolates could in part be attributed to the high proportion of multi drug resistant methicillin resistant isolates that prevail in hospital setting (Al-Masaudi, et al, 1991; Cookson et al, 1991; Akimitsu et al., 1999; Irizarry et al, 1996; Lambert, 2004; Suller and Russell, 1999).

As they have more subtle targets, resistance to antibiotics is reflected as a distinctive rise in the MICs that could be easily detected by two fold serial dilution procedure for determination of MIC. However, this dilution pattern may not be suitable for detection of the slight changes in MICs attributed to loss or reduced sensitivity to one of the multiple targets of the given biocide. Therefore, arithmetic rather than geometric progression was used for determination of MIC of biocides, and subsequently comparison based on the log MIC values that seem to be more reasonable and was proposed.

Pearson's correlation coefficients between MIC-MIC, log MIC-MIC, and log MIC-log MIC for antibiotic- biocide pairs were determined and compared. The three values for each antibiotic-biocide pair were compared for agreements and discrepancies (odds) with respect to the significance or non significance, of the potential correlation (table 13). Out of the three comparisons, log MIC for antibiotic versus MIC for biocide seemed to be the most representative for trends with the least number of odds, followed by log MIC-log MIC comparison and finally came the MIC-MIC comparison. Yet, all three comparisons, with few exceptions, confirmed significant correlations.

For hospitals' isolates and except for chlorhexidine and povidone-iodine and occasionally with phenyl mercuric nitrate, a positive correlation was found between resistance to the tested biocide and most of the tested antibiotics. For non-hospital isolates only cetrimide resistance correlated with antibiotic resistance. Collectively for the combined isolates, however, the positive correlation was found between antibiotics and biocides except for chlorhexidine and povidone-iodine.

Another way to correlate biocide resistance with antibiotic resistance was to compare the percentage of isolates resistant to individual antibiotics for biocide resistant and susceptible isolates. Except for chlorhexidine and povidone-iodine, the frequency of antibiotic resistance was higher among biocide resistant than biocide susceptible isolates. Surprisingly, the frequencies of antibiotic resistance were higher among the biocide sensitive than in the biocide resistant isolates for chlorhexidine or povidone-iodine.

In conclusion, the current study demonstrates high frequency of resistance of *Staphylococcus* isolates from hospital sources to some common antimicrobial agents and a positive correlation between biocides and antibiotics resistance was proven. Exceptions from this were with chlorhexidine and povidone-iodine, where resistance did not correlate with resistance to antibiotics. The study demonstrates the validity of the proposed approch for correlating antibiotic and biocide resistance based on MIC data and also demonstrates high antibiotic and biocide resistance in hospital setting.

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طريقة تحليل مقترحة لاستخدام التركيزات الدنيا المثبطة للنمو في المكورات العنقودية لايجاد علاقات الارتباط بين المقاومة للمضادات البكتيرية العلاجية وقاتلات الجراثيم

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استهدفت الدراسة محاولة الربط بين نقص حساسية المكورات العنقودية لقاتلات الجراثيم والمقاومة للمضادات الميكروبية العلاجية. وقد استخدم في الدراسة عزلات مكورات عنقودية من مصادر مختلفة شملت ٤٧ عزلة سريرية، ٦٤ عزلة من وسط المستشفى، و ٣٣ عزلة بيئية من خارج المستشفى. تم تعيين التركيزات الدنيا المثبطة لنمو العزلات لمجموعة من المضادات الميكروبية العلاجية وأخرى من قاتلات الجراثيم باستخدام طريقة التخفيف في الاجار حيث استخدمت اغتمدت زيادة عددية منتظمة في حالة قاتلات الجراثيم بدلا من الزيادة الهندسية المتبعة للمضادات الحيوية. وتم التحليل الاحصائي لمقارنة التركيزات الدنيا المثبطة او الاشتقاق اللوغاريتمي لها او بهجين منها لايجاد درجة الارتباط بين افراد المجموعتين بايجاد معامل بيرسون. كما تم تحليل النتائج بطريقة أخري اعتمدت على تصنيف أولي بحسب الحساسية لقاتلات الجراثيم كل على حده الى مجموعتين حساسة ومقاومة ثم تقدير نسبة العزلات المقاومة للمضادات العلاجية في كل مجموعة ومقارنة نتائج الطريقتين. وبالاضافة الى ارتفاع نسبة المقاومة لكل من مجموعتي المضادات في عزلات المستشفى فقد اظهرت وجود علاقة ارتباط قوية بين درجة المقاومة للمضادات العلاجية ونقص الحساسية لقاتلات الجراثيم وبخاصة في عزلات المستشفى والعزلات السريرية وأن افضل مقياس لهذه العلاقة هو مقارنة التركيز المثبط لقاتلات الجراثيم مع لوغاريتم التركيز المثبط للمضادات العلاجية. وفيما عدا الكلورهكسيدين، و بوفيدون الايودين ارتبطت ايجابيا المقاومة لقاتلات الجراثيم (كلوروكريزول، كلوريد البنزالكونيوم،ستريميد،نترات فنيل الزئبق، بروميد الايثيديم) بالمقاومة للمضادات العلاجية فيما عدا فانكومايسين (وبعض الاستثناءات مع تتراسيكلين وسبروفلوكساسين). فيما يتعلق بالعزلات البيئية فقد كانت درجة المقاومة منخفضة نسبيا ووجدت علاقة ارتباط ايجابية بين المقاومة للمضادات العلاجية وكل من كلوريد البنزالكونيوم وستريميد