THE LINKAGE BETWEEN ANTIBIOTICS AND BIOCIDES RESISTANCE IN SOME GRAM POSITIVE COCCI

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2008

بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنا إلا ما علمتنا انك أنت العليم الحكيم

صدق الله العظيم

سورة البقرة – آية ٣٢

This effort is dedicated

to

the soul of Dr.Mousa Khalid Okasha,

my parents,

beloved wife and

sweet son, Mohamed .....

العلاقة بين مقاومة المضادات الحيوية و قاتلات الجراثيم في بعض مكورات البكتريا موجبة الجرام

رسالة مقدمة من

الصيدلاني

أحمد علوان محمد الحسني

(بكالوريوس العلوم الصيدلية / ٢٠٠٣)

للحصول على درجة

الماجستير في العلوم الصيدلية

(ميكروبيولوجي)

قسم الميكروبيولوجي

كلـــــية الصيدلـــــة

جامـعة الزقازيــق

٢٠٠٨

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٢٠٠٨

Discussion

Although it is generally agreed that the main cause of antibiotic resistance is the excessive and misuse of antibiotics, some scientists suggest that widespread use of biocides, in hospital, domiciliary, industrial, and other settings, may be a contributory factor (**Levy 1998; Russell *et al.*1998, 1999; Levy 2002**). The emergence of bacterial resistance to biocides and the possible linkage between biocide and antibiotic resistance is a major topic of discussion and concern **(Maillard, 2005)**. The present study aimed to contribute to the current debate concerning the mutual role of increased resistance to either biocides or antimicrobial chemotherapeutic agents on the resistance of the other agent and to find any correlation between resistance to either agent.

Gram positive cocci were targeted in the present study, where, one hundred and fifty clinical specimens, collected during one year period from May 2005 to March 2006, yielded 31.33% *Staphylococcus* spp. and 30% *Enterococcus* spp. **(Table 4)**. These results are in agreement with other data from various parts of the world in the impact of these microorganisms in clinical settings (**Shamsuzzaman *et al.*, 2003; Johnson *et al.*, 2003; Sattar *et al.*, 2005; Gebre-Sealsssie, 2007; Huang *et al.*, 2007).** Additionally, out of 80 pus specimens collected in this study, 42.5% and 12.5% were identified as *Staphylococcus aureus* and CoNS, respectively. These results reflect the role of *Staphylococcus* spp. in pyogenic infections and are in agreement with that of other workers (**Christou *et al.*, 2004; Mulu *et al.*, 2006; Anguzu and Olila, 2007).** On the other hand, our study also demonstrated the prevalence of

Staphylococcus spp. in environmental surfaces of ICU. These results are in accordance with those previously reported by **Asoh *et al.* (2005); Shobha *et al.* (2005)** and **Brady *et al.*(2007).**

This study showed that, about 32% and 28% of *Staphylococcus* isolates from clinical and hospital environment specimens, respectively, were methicillin resistant (**Table 9**). In agreement with **Kesah *et al.* (2003),** methicillin resistance was detected in 15% of the 1440 clinical *Staphylococcus* isolates tested in eight large hospitals in Africa and Malta, during a period from 1996 to 1997. They found that, the rate of MRSA was relatively high in Nigeria, Kenya, and Cameroon (21-30%), and below 10% in Tunisia, Malta, and Algeria. Higher methicillin resistance rate, in Egypt, was reported by **El Kholy *et al.* (2003)** and **Ashour and El-Sharif (2007).** Moreover, **McDonald *et al.* (2004**) found that, oxacillin resistance rates among *Staphylococcus aureus* and CoNS, collected in Taiwan, were 60% and 80%, respectively. Additionally, **Székely *et al.* (2008)** detected methicillin-resistance in 50% of 423 *Staphylococcus aureus* strains identified from clinical specimens collected between 2004 and 2005 in Romania**.**

Furthermore**, Claesson *et al.* (2007)** found oxacillin resistance among 47% of CoNS isolates collected in Denmark, Finland, Norway and Sweden. Our clinical *Staphylococcus* isolates showed resistance rate of 70% for both penicillin G, and ampicillin. This high resistance rate is in line with other workers from various parts of the world. **Randrianirina *et al.* (2007)** found that, resistance rate of clinical *Staphylococcus aureus*, collected in Madagascar during a period from 2001 to 2005, to penicillin G was 91.2%**.** In Ethiopia resistance rates of clinical *Staphylococcus aureus* isolates to penicillin and ampicillin were 95% and 93%, respectively (**Mulu *et al.*, 2006).** In Uganda, 97% of clinical

Staphylococcus aureus isolates, were resistant to ampicillin (**Anguzu and Olila, 2007).**

However, clinical *Staphylococcus* isolates showed susceptibility rates of 70% for cefotaxime, ceftriaxone and cefepime and 72.3% for cefoperazone. High susceptibility to cephalosporins has been previously reported. In Lebanon**, Kanj *et al.* (2001**) reported that, all the oxacillin susceptible *Staphylococcus* clinical isolates were susceptible to cefepime**.** While, **Ahmed *et al.* (2002)** showed that, almost all of *Staphylococcus aureus* clinical isolates, collected in Pakistan, were sensitive to cefotaxime. Moreover, **Karlowsky *et al.* (2002)** found that, among clinical isolates of methicillin-susceptible *Staphylococcus aureus*, ceftriaxone resistance was 0.1 - 0.3% per year from 1996 to 2000 in USA.

In the current study, clinical *Staphylococcus* isolates showed resistance rate of 17% to gentamicin. This relatively low rate of resistance is consistent with other studies. **Ma *et al.* (2003)** found that, 16.1% of *Staphylococcus aureus* clinical isolates in China were resistant to gentamicin. In addition, **Anguzu and Olila (2007)** showed that 12.5% of *Staphylococcus aureus* clinical isolates from Uganda were resistant to gentamicin. Moreover, clinical *Staphylococcus* isolates in this study showed resistance rate of 32% to streptomycin which is comparable to other studies. **Tenssay (2000)** found that 49% of *Staphylococcus aureus* clinical isolates in Ethiopia were resistant to streptomycin. However, 67% of clinical *Staphylococcus aureus* isolates in Pakistan were resistant to streptomycin **(Sattar *et al.*, 2005).**

Furthermore, in the present study, ciprofloxacin resistance was detected in 19% of clinical *Staphylococcus* isolates. In agreement with **Ahmed *et al.* (2002)**, 26% of clinical *Staphylococcus aureus* isolates,

collected in Pakistan, were resistant to ciprofloxacin**.** In China , the resistance rate of clinical *Staphylococcus aureus* isolates to ciprofloxacin was 11.2% (**Ma *et al.*, 2003).**

Chloramphenicol resistance was found only in 13% of clinical *Staphylococcus* isolates in our study. Low chloramphenicol resistance rate was also reported by other studies. **Rohani *et al.* (2000)** found that, the resistant rate of clinical *Staphylococcus aureus* isolates, collected in Malaysia, to chloramphenicol was 8.5%. In addition, the resistance rate of *Staphylococcus aureus* clinical isolates, collected in Madagascar from 2001 to 2005, to chloramphenicol was 11.7% (**Randrianirina *et al.*, 2007).**

In the present study, tetracycline resistance was detected in 33% of clinical *Staphylococcus* isolates. However, higher resistance rate was detected by **Randrianirina *et al.* (2007)** who found that 51.4% of clinical *Staphylococcus aureus* isolates, collected in Madagascar from 2001 to 2005, were resistant to tetracycline**.** In addition, **Mulu *et al.* (2006)** showed that, resistance rates of *Staphylococcus aureus* clinical isolates, collected in Ethiopia, to tetracycline was 57%.

All of *Staphylococcus* and *Enterococcus* isolates tested in this study were susceptible to vancomycin. This high susceptibility may be due to its infrequent use in Zagazig University hospitals. These results are in accordance with other studies. All clinical enterococcal isolates tested during 2004 to 2005 in Spain were sensitive to vancomycin (**Causse *et al.*, 2006).** Moreover, no vancomycin resistance was observed among MRSA clinical isolates obtained from 1999 through 2006 in USA (**Holmes and Jorgensen, 2008).** In addition, **Cifuentes *et al.* (2005)** showed that, all of *Staphylococcus aureus* clinical isolates in Colombia were sensitive to

vancomycin. Furthermore, **Abbassi *et al.* (2008)** found that, all of clinical *Enterococcus* isolates studied in Tunisia were sensitive to vancomycin.

In contrast, *Staphylococcus aureus* with intermediate resistance to vancomycin (VISA) was first observed in a strain isolated from a hospitalized patient in Japan **(Hiramatsu *et al.*, 1997)**. While, in the United States, four cases of VISA were reported between 1997 and 1999 **(Smith *et al.*, 1999; Sieradzki, 1999)**. Furthermore, the first *van*A-mediated, vancomycin-resistant *Staphylococcus aureus* (VRSA) strain was isolated in a Michigan hospital in 2002 (**Chang *et al.*, 2003**), six additional occurrences were also reported (**Weigel *et al.*, 2003**). In the same line, 20% of Enterococci obtained by **Devi *et al.* (2002)** from various clinical specimens in India were resistant to vancomycin**.** Additionally, **Acharya *et al.* (2007)** found that**,** 13% and 17% of *Enterococcus faecalis* and *Enterococcus faecium*, clinical isolates collected in Nepal during a period from 2002 to 2003, were resistant to vancomycin**.**

Clinical *Enterococcus* isolates, tested in this study, showed high susceptibility rate (98%) for each of penicillin G and ampicillin. High susceptibility rates were also detected by other workers. **Saunders and Bodonaik (2006)** showed that, susceptibility rate of clinical *Enterococcus* isolates, collected from Jamaica, to ampicillin was 89%. Moreover, susceptibility rate of clinical *Enterococcus* isolates, collected in Spain during a period from 2004 to 2005, to ampicillin was 98% (**Causse *et al.*, 2006).** Additionally, almost, all clinical *Enterococcus* isolates, collected in Australia during 2005, were susceptible to ampicillin (**Christiansen *et al.*, 2007)**.

Furthermore, our work revealed that clinical *Enterococcus* isolates showed resistance rates of 18% and 93% for gentamicin and streptomycin, respectively. However, **Calderón-Jaimes *et al.* (2003)** found that, resistance rates of clinical isolates of *Enterococcus* spp., in Mexico, were low for gentamicin (32%) and high for streptomycin (57%). In the same line, **Causse *et al.* (2006)** showed that, resistance rates of clinical *Enterococcus* isolates, collected during a period from 2004 to 2005 in Spain, were low for gentamicin (24%) and high for streptomycin (64%).

Furthermore, in our study, clinical *Enterococcus* isolates were highly susceptible (91%) to ciprofloxacin. In contrast, high resistance rate was obtained by **Acharya *et al.* (2007)** who found that**,** ciprofloxacin resistance rate of *Enterococcus faecium* clinical isolates, collected in Nepal during a period from 2002 to 2003, was 63%. Additionally, ciprofloxacin resistance was detected in 80% of clinical *Enterococcus* isolates collected in Taiwan during a period from 2004 to 2005 (**Huang *et al.*, 2007).** In addition, **Abbassi *et al.* (2008)** showed that, ciprofloxacin resistance rate of clinical *Enterococcus faecium* isolates, collected in Tunisia, was 79%.

In the present study, clinical *Enterococcus* isolates showed high susceptibility (91%) to chloramphenicol. High susceptibility rate was also reported by **Titze-de-Almeida *et al.* (2004)** who found that, all of the *Enterococcus faecium* clinical isolates, collected in Brazil, were sensitive to chloramphenicol. Moreover, our clinical *Enterococcus* isolates were highly susceptible (91%) to tetracycline. In contrast, high resistance rates were obtained by **Titze-de-Almeida *et al.* (2004)** who found that, the resistance rates of clinical *Enterococcus faecalis* and *Enterococcus faecium* isolates for tetracycline were 71% and 78%, respectively**.** In addition, 92% of *Enterococcus faecalis* clinical isolates

from Jamaica were tetracycline resistant (**Saunders and Bodonaik, 2006)**. Moreover, **Abbassi *et al.* (2008)** detected tetracycline resistance in 43% of *Enterococcus faecium* clinical isolates from Tunisia.

The increased usage of products containing low concentrations of commonly used biocides, such as phenolics and cationic compounds, has raised some concerns about their overall efficacy, but also about the possible emergence of microbial resistance. Indeed, there are now multiple laboratory reports about the emergence of bacterial resistance to biocides, often as a result of exposure to a lower (sublethal) concentration. The emergence of bacterial resistance to biocides is not a new phenomenon and has been described since the 1950s, particularly with products containing a cationic biocide. Moreover, the emergence of bacterial resistance to biocides to low (inhibitory) concentrations has been widely reported, mainly from laboratory studies, but also from environmental investigations. Low to intermediate levels of resistance have been observed in most cases, although from time to time high-level resistance has been reported **(Maillard, 2005)**.

Our data revealed that about 28 % and 100% of clinical *Staphylococcus* and *Enterococcus* isolates, respectively, were resistant to chlorocresol (MICs ≥ 250 µg/ml). Possible mechanism of resistance to chlorocresol can be due to enzymes transforming the bactericide to a non-toxic form **(Cloete, 2003)**. Moreover, clinical *Staphylococcus* isolates showed resistance to benzalkonium chloride, chlorhexidine, cetrimide and ethidium bromide (MICs ≥ 4.0, 2.0, 8.0 and 10 µg/ml, respectively) at rates of about 60%, 21%, 19%, and 13%, respectively. On the other hand, clinical *Enterococcus* isolates were completely resistant to benzalkonium chloride, cetrimide and chlorhexidine (MICs ≥ 4.0, 8.0 and

2.0 μg/ml, respectively), and showed resistance to ethidium bromide (MICs ≥ 10 μg/ml) at rate of about 91%. *Staphylococcus* isolates from clinical and food sources were found to carry multidrug-resistant plasmids containing the *qacA, qacB, qacC,* and *qacD* genes, which encode multidrug efflux pumps that mediate non-susceptibility to cationic biocides, such as chlorhexidine gluconate, ethidium bromide, and quaternary ammonium compounds **(Sheldon, 2005).**

In the present study, 51% and 98% of clinical *Staphylococcus* and *Enterococcus* isolates, respectively, were resistant to phenyl mercuric nitrate (MICs ≥ 0.2 μg/ml). Resistance to mercury was found to be plasmid borne, inducible, and may be transferred by conjugation or transduction. Inorganic mercury (Hg^{2+}) and organomercury resistance is a common property of clinical isolates of *Staphylococcus aureus* containing penicillinase plasmids. Plasmids conferring resistance to mercurials are either narrow spectrum, specifying resistance to Hg^{2+} and to some organomercurials, or broad-spectrum, with resistance to these compounds and to additional organomercurials **(McDonnell and Russell, 1999).** Detoxification of organomercurials (Om^r) is mediated by an organomercurial lyase which cleaves the carbon-mercury bonds to produce Hg^{2+} ions that are volatilized in turn by the reductase **(Lyon and Skurray, 1987).**

In the light of our investigation, it seems that clinical staphylococci and enterococci and environmental staphylococci isolated from ICU in Zagazig university hospital showed higher resistance rates to most of the tested antimicrobial chemotherapeutic agents and biocides compared to environmental non-hospital isolates (**Tables 15 and 23**). This is may be due to the high usage of antimicrobials in hospitals compared to non-