Oxacillin resistance in *Staphylococcus* clinical isolates from Havana City Hospitals, Cuba

Aлина Чебла, Мария Эспино,* Илеана Гонсалес, Илеана Отеро, Марилейс Лемус, Леонарда Гонсалес и Карлос Вальин.

Microbiology Laboratory, Department of Biotechnology, Centre of Pharmaceutical Chemistry. 25th Ave. 158 street, Cubanacán, Playa, P.O. Box 414, Havana City *Laboratory of Antibiotic Resistance, Gyneco-Obstetric Hospital "America Arias", G street and Line street, Vedado, Revolution Place, P.O. Box 1400, Havana City, Cuba.


Palabras clave: resistencia a oxacilina, *Staphylococcus*, MRSA, resistencia cruzada a antibióticos.

Key words: oxacillin resistance, *Staphylococcus*, MRSA, antibiotic cross-resistance.

RESUMEN. Durante la última década las bacterias Gram positivas han emergido gradualmente como la causa más frecuente de enfermedades nosocomiales. *Staphylococcus aureus* meticilina-resistente (MRSA) surgió como un patógeno nosocomial y se ha diseminado por todo el mundo desde mediados de 1970. La naturaleza general de este problema continúa a comienzos de 1990 y se ha planeado que todos los hospitales de diferentes tamaños ya lo están enfrentando. La frecuencia de MRSA parece incrementarse en Estados Unidos, Japón, España, Bélgica y otros países, sin embargo, sólo unos pocos agentes antimicrobianos están disponibles para el tratamiento de este tipo de infecciones como consecuencia de su múltiple resistencia a antibióticos. En Ciudad de La Habana no existían datos sobre la frecuencia de aislamiento de MRSA. No obstante, los índices de morbilidad y fallos en la quimioterapia para el género *Staphylococcus* han sido reportados y son significativos. Se llevaron a cabo un estudio para determinar la frecuencia de MRSA en cuatro hospitales de la Ciudad usando el método de tamizaje en agar-oxacilina. Fueron estudiados la presencia de β-lactamasa y los valores de mínima concentración inhibitoria contra el oxacilina. También se determinaron los patrones de resistencia de los MRS contra otros antibióticos. El 30,7 % de las cepas de *Staphylococcus* fueron oxacilina resistentes con una resistencia cruzada amplia contra otras familias de antibióticos. La β-lactamasa estuvo presente en el 26,3 % de todas las cepas, donde el 83 % de este grupo fue MRSA. No se encontró resistencia a la vancomicina en ninguna cepa. MRSA representó el 6 % de todas las cepas estudiadas.

ABSTRACT. During the past decade Gram positive bacteria have gradually emerged as the most frequent causes of nosocomial disease. *Staphylococcus aureus* meticillin-resistant (MRSA) emerged as a nosocomial pathogen and has disseminated worldwide since the mid-1970s. The widespread nature of this problem continues into the early 1990s and it has been suggested that hospitals of all sizes are facing the MRSA problem. The frequency of MRSA appears to be increasing in the United States, Japan, Spain, Belgium and other countries but only a few antimicrobial agents are available for treatment of this kind of infection as a consequence of their multiple antibiotic resistance. In Havana City, there were no data about the frequency of isolation for MRSA. However the morbidity indexes and failures in the chemotherapy against *Staphylococcus genusc* have been reported and it is significant. A study was carried out in order to determine the frequency of MRSA in four hospitals from that City using the oxacillin agar screen method. The presence of β-lactamase and the values of their minimum inhibitory concentration (MIC) against oxacillin were studied. Also it was determined the resistance patterns of MRSA against other antibiotics. The 30,7 % of *Staphylococcus* strains were oxacillin resistant with an extensive cross-resistance against other families of antibiotics. β-lactamase was present in the 26,3 % of all the strains where 83 % of this group were MRS. No vancomycin resistance was found in any strain. Meticillin resistant *Staphylococcus aureus* (MRSA) represented the 6 % of all the strains tested.

INTRODUCTION

During the past decade Gram positive bacteria have gradually emerged as the most frequent causes of nosocomial disease. *Methicillin-resistant S. aureus* (MRSA) was first described in England in 1961, emerged as a nosocomial pathogen in Europe and subsequently in Australia, and have disseminated worldwide since the mid-1970s. The widespread nature of this problem continues into the early 1990s. It has been suggested that hospitals of all sizes are facing the MRSA problem.

Coagulase-negative *Staphylococcus* (CoNS) species is a major component of the normal microflora of humans. Over the last two decades there has been an increase of CoNS in the infections being the methicillin resistant *Staphylococcus* (MRS) from 60 to 90 percent of all isolates. The frequency of MRS appears to be increasing in the United States, Japan, Spain, Belgium and other countries. A few antimicrobial agents are available for treatment of this kind of infections as a consequence of their multiple antibiotic resistance.

In Havana, there were no reports about the frequency of MRSA isolation, however the morbidity indexes and failures in the chemotherapy against this group have been significant. A study was carried out in order to determine the appearing fre-
Materials and methods

Organisms tested

Two hundred two clinical isolates of Staphylococcus were studied by the oxacillin agar screen. The MIC values were determined for the MRS strains by the standard broth microdilution method. In addition, β-lactamase presence was determined in all of them and they were classified according to the procedure described in Manual of Clinical Microbiology (morphological characteristics and biochemical tests).

The clinical isolates were obtained from several sources (Gynecological and Obstetric Hospital “America Aria”, Clinic-Chirurgical Hospital “Joaquin Albarán”, General Hospital “Carlos J. Finlay” and Pediatric “Juan M. Márquez”, all in Havana City. Bacterial stocks were kept frozen at -70°C in 50% sterile glycerol containing medium and supplemented with oxacillin if the strains were MRS.

Strains Staphylococcus aureus 1870, Penicillinase-Resistant Penicillin (PRP) sensitive and Staphylococcus aureus 1426 PRP resistant were generously provided by Dr. Eddie Power from St. Thomas Hospital, London. Staphylococcus aureus ATCC 25923 was used for quality control purposes as recommended by the National Committee for Clinical Laboratory Standards.

Antimicrobial susceptibility tests

Standardized disk diffusion method was used to determine the susceptibility of MRS isolates to the following antibiotics: penicillin G (P), oxacillin (OX), methicillin (MET), cephalzin (KZ), cephalothin (KF), cefotaxime (CTX), ceftriaxone (CRO), ciprofloxacin (CIP), ofloxacin (OFX), erythromycin (E), clindamycin (CLR), clindamycin (DA), gentamicin (CN), tetracycline (TE), sulfamethoxazole-trimethoprim (SXT), chloramphenicol (C) and vancomycin (VA) (Unipath-Oxoid, UK).

Staphylococcal clinical isolates were inoculated onto Mueller-Hinton agar plates containing oxacillin (Smith Kline Beecham Pharmaceuticals, UK) at 6 mg/mL plus 4% NaCl and incubated at 35°C for 24 h. Isolates that grew on this medium were classified as MRS. Moreover, analysis of antimicrobial susceptibility patterns of MRS or MRSA was carried out in order to characterize much better the strains.

Determination of MIC

Each isolate MRS was tested by the standard broth microdilution test with an inoculum of approximately 5·10^8 CFU/mL salt-supplemented (2%) Mueller-Hinton broth.

The antibiotics oxacillin (Smith Kline Beecham Pharmaceuticals, UK) and vancomycin (IMEFA, Cuba) were used between 0.5 and 256 mg/mL.

β-Lactamase detection

Qualitative β-lactamase assay using nitrocefin (Oxford, UK) as the substrate was performed according to NCCLS guidelines in all the strains.

Results and discussion

A total of 202 clinical isolates were tested to determine resistance to PRPs and 62 strains (30.7%) were MRS according to the agar screen plate. From the total of strains (202), twelve Staphylococcus aureus were classified as MRSA (6%) while 37 strains of Staphylococcus epidermidis (18.3%) were methicillin resistant. Just 3 strains of S. saprophyticus (1.48%) were MRS and within the group of non-classified strains there were nine strains MRS (4.45%).

ColNS methicillin resistant have been deeply studied and they reach current frequencies of 60-90%. The percents show these are much lower values than the ones found in the literature summing S. epidermidis and S. saprophyticus strains (19.8%). These results could indicate that the frequency of methicillin resistant Staphylococcus epidermidis does not constitute today a serious problem in clinic for the country. It could be related to the availability and use of this antibiotic, which is quite limited at the moment, exerting a control in the resistance rates. However, the authors should keep surveillance over this group. S. epidermidis has been considered since 1995 as an emergent pathogen. The number of isolates reported as causal agents of pathologies is gradually increasing and with it the number of strains transporting multiple resistance problems.

The scores reported for Staphylococcus aureus (6%) are inferior to the findings in other countries, which have risen 5 to 40% from 1986 to 1992. S. aureus is taking the second place in terms of methicillin resistance incidence but presents much more importance in the morbidity indexes as a causal agent in pathologies for newborns and children. The reasons why the percents are lower than the ones in the literature are the same exposed before for S. epidermidis.

MRSA has been extended from the large teaching hospitals into smaller hospitals units. The majority of the strains are resistant to a wide range of antibiotics being glycopeptides the therapy of choice. Initially there was the possibility of emergence of MRSA vancomycin resistant taking into account evidences given by transfer experiments of vancomycin resistance genes from Enterococcus to Staphylococcus. Recently have been reported two MRSA vancomycin resistant strains. Now the authors are facing the named methicillin vancomycin resistant strains without treatment. Fortunately, all MRSA strains found in the study were sensitive to vancomycin.

Vancomycin was introduced in Cuba by 1980 and was included in the group of controlled antibiotics in the national policy. The country is receiving vancomycin as a donation from other countries which help with some drugs the National Health System. It is very important to inform and advice a medical personnel about the risks implied and the indiscriminate use of this antibiotic creating a selective pressure for vancomycin resistant strains.

The antibiotic susceptibility spectrum from MRS isolates against other families of antibiotics showed a wide cross resistance (Table 1) and MIC values for OXA were equal or superior to 4 mg/mL and equal or lower to 4 mg/mL for VA.

The levels of OXA-resistance were directly proportional to the cross-resistance percents and also it did influence the sensitivity of Bauer-Kirby method for the detection of MRS. The 100% of MRS strains detected by oxacillin screen method showed P resistance by Bauer-Kirby method, however just 46 (74.2%) to 47 (75.8%) of 62 strains (OXA screen agar) were classified as MRS or OXA resistant using Bauer-Kirby method. Having a look at the data obtained (Fig. 1) it can see the relationship between the MIC values and the sensitivity for detection of MRS using Bauer-Kirby. The 42.8% of strains with MICs values around 4 mg/mL (closer to borderline resistance for OXA) were not detected, while the 71.4% of the strains having MIC values between 8 and 16 mg/mL were right detected and the 100% of MRA were absolutely diagnosed for MIC values between 32 and 256 mg/mL.
here we can evaluate how important the method to be employed for meticillin resistance detection is, the lack of enough sensitivity of Bauer Kirby method for this purpose and how the strain resistance levels could affect the results in the lab. Also on this figure can see a relationship between the OXA resistance levels and the cross-resistance phenomenon, taking higher percents for strains with maximum MIC values.

The results show resistance in the 50% of the MRS strains to four cephalosporins, in the 17.74% to three macrolides (MLS) and in the 38.7% to penicillins, cephalosporins, at least one macrolide, CN and at least one of group (TE, SXT and C). The antibiotics confronting higher resistance percents were CTX (72.58%) and CRO (52.25%), E with 59.67%, CN with 53.22%, TE with 48.38 and SXT, 70.96%.

However MRS were very sensitive to fluoroquinolones (more than 89%), so they could be a true option in the treatment of adult patients. Fluoroquinolones are of extremely limited use in the country because of its recent introduction and the national regulations. Another reason is the nature of the resistance mechanisms developed in bacteria for this family, which is not plasmid mediated and it makes more difficult the resistance genes spread. Nevertheless Cuban hospitals are receiving fluoroquinolones at the moment through donations from other countries. Sometimes the antibiotic available must be use a consequence of the lack of other antibiotics on detriment of the regulations. Physicians should be very careful with fluoroquinolones because even with a national policy it is difficult to avoid the use of some antibiotics, mainly when are dealing with human lives.

Exist different resistance mechanisms explaining the oxacillin resistance phenotype in bacteria. When a new penicillin binding protein (PBP2a) is present (classic resistance phenotype) the strains shows cross resistance with other b-lactams and multiple resistance to non-3-lactams. They are divided into homogeneous and heterogeneous groups and are mec gene encoded but the heterogeneous group shows borderline resistance. The results indicate that the total of the strains classified using oxacillin screen agar probably presents this resistance mechanism.

β-lactamase was initially considered to be involved in the mechanism of resistance to meticillin in MRSA. However, in the 1980’s, such resistance was shown to be due to the production of PBP-2a with a low affinity for meticillin. It was reported that the production of PBP-2 was essential for resistance to meticillin but production of β-lactamase had a major effect on the MIC.

In the study, β-lactamases were present in 53 strains of 202 (26.3%), where 44 (83%) were MRS and 9 (17%) were no MRS. Unfortunately the authors did not study the levels of β-lactamases production but they can state that all of them had MIC values over 4 mg/mL.

However in vitro results, we can not affirm that MRS strains sensitive to some β-lactams will be susceptible to these antibiotics during therapy. NCCLS guidelines recommend that MRS should be reported

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>KZ</th>
<th>KF</th>
<th>CTX</th>
<th>CRO</th>
<th>E</th>
<th>DA</th>
<th>CLR</th>
<th>CN</th>
<th>TE</th>
<th>SXT</th>
<th>C</th>
<th>CIP</th>
<th>OFX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (%)</td>
<td>56.5</td>
<td>51.6</td>
<td>72.6</td>
<td>82.3</td>
<td>59.7</td>
<td>22.6</td>
<td>58.1</td>
<td>53.2</td>
<td>48.4</td>
<td>71</td>
<td>45.2</td>
<td>11.3</td>
<td>8.1</td>
</tr>
</tbody>
</table>

**Table 1. Cross resistance of MRS isolates against other families of antibiotics.**

**Cephalosporins**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>KZ</th>
<th>KF</th>
<th>CTX</th>
<th>CRO</th>
<th>E</th>
<th>DA</th>
<th>CLR</th>
<th>CN</th>
<th>TE</th>
<th>SXT</th>
<th>C</th>
<th>CIP</th>
<th>OFX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (%)</td>
<td>56.5</td>
<td>51.6</td>
<td>72.6</td>
<td>82.3</td>
<td>59.7</td>
<td>22.6</td>
<td>58.1</td>
<td>53.2</td>
<td>48.4</td>
<td>71</td>
<td>45.2</td>
<td>11.3</td>
<td>8.1</td>
</tr>
</tbody>
</table>

**MLS Macrolides**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>KZ</th>
<th>KF</th>
<th>CTX</th>
<th>CRO</th>
<th>E</th>
<th>DA</th>
<th>CLR</th>
<th>CN</th>
<th>TE</th>
<th>SXT</th>
<th>C</th>
<th>CIP</th>
<th>OFX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (%)</td>
<td>56.5</td>
<td>51.6</td>
<td>72.6</td>
<td>82.3</td>
<td>59.7</td>
<td>22.6</td>
<td>58.1</td>
<td>53.2</td>
<td>48.4</td>
<td>71</td>
<td>45.2</td>
<td>11.3</td>
<td>8.1</td>
</tr>
</tbody>
</table>

**Groups according to oxacillin MIC values**


**Fig. 1. Cross resistance of MRS strains against other antibiotic families.**
as resistant to all β-lactam family without taking into account in vitro tests results. The majority of the cases with this kind of infection have responded very poorly to β-lactam therapy.

Differences were found in the distribution of resistance rates among hospitals or by hospital type. The higher resistance percentage was in the Pediatric Hospital "J.M. Marquez" with 34%. This could be due to the fact that sepsis by Staphylococcus in quite often in children and new-borns. On the other hand, this family of antibiotics is of a wide use on this patient group due to their low toxicity and spectrum activity. "J. Albarrán" with 31.6% and "C.J. Finlay" with 28.6% followed this hospital. Both of them are Clinical-Surgical Hospitals where Staphylococcus has a high incidence as infectious agent in surgical wounds.

The lowest records were from "America Arias" Hospital with 16.6%. The latest is a Gyneco-Obstetric Hospital where they have a high incidence of Staphylococcus genus but a very good antibiotic policy in order to control the antibiotic resistance within the institution. They also have developed a program to monitoring the presence of MRS and MRSA and introduced combined therapy using aminoglycosides and β-lactams antibiotics together, based on the information of their microbiological map and the most frequent resistance patterns in order to avoid the spread of resistance genes.

CONCLUSIONS

The resistance records are a result of the advantages obtained from the application of a national policy for the rational use and consumption of antibiotics, directed to control the resistance developed in bacteria, to protect the antibiotics and also to improve the quality of the patient medical treatment. Oxacillin resistance is not a problem yet in clinical isolates (MRS). MRSA is not a threat in the hospitals at the moment, let alone vancomycin resistance. However the oxacillin resistance rates found in S. epidermidis were three times higher than in S. aureus and this species is taking more and more importance as an emergent pathogen in clinic today. It suggests the necessity of a systematic surveillance of oxacillin and vancomycin resistance in Cuban centres to prevent the appearing or resistance increase on this species.

All in all, this study provided preliminary data on recent MRS and MRSA prevalence trends in the capital showing variation among hospitals. Precautions must be taken in order to avoid an increase of the oxacillin resistance in the isolates. A large multicentric in vitro study has been planned directed to the specific detection of MRS and their epidemiological typing. A national surveillance system will be established in Cuba based on the results from this multicentric study. This system will monitor trends of MRS prevalence and the efficacy of the updated control strategies.

World-wide surveillance for resistance will help identify factors leading to the emergency and spread of resistance isolates in all over the countries and may uncover new resistance genes that will alert the world to impending problems.

BIBLIOGRAPHY