Prevalence and antibiotic susceptibility profile of methicillin resistant *Staphylococcus aureus* in Accra, Ghana

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Abstract

Over the last four decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has spread throughout the world and become highly endemic in many geographical areas. This pathogen causes severe morbidity and mortality in hospitals worldwide. MRSA is also considered a major community acquired pathogen throughout the world. MRSA is implicated in serious clinical conditions such as bacteremia, pneumonia, and intra-abdominal infection. The objective of this study was to determine the prevalence of MRSA in Accra, Ghana, and also to determine its antibiotic susceptibility profile. Two hundred and fifty *Staphylococcus aureus* isolates from routine microbiological specimens were collected from five hospitals in Accra. MRSA screening assay was used to screen for MRSA. Disc diffusion method (Kirby-Bauer) was used to determine the susceptibility of the MRSA. The MRSA screening assay, which is very close to the polymerase chain reaction in terms of specificity and sensitivity, showed that 84 of the 250 isolates were MRSA, giving a prevalence rate of 33.6%. MRSA strains were susceptible to erythromycin; 63 out of the 84 MRSA isolates were susceptible representing 75%. This was followed by gentamicin 46 (54.7%), cotrimoxazole 35 (49%), cefuroxime 33 (38%), flucloxacillin 24 (28.6%), and ampicillin 13 (15.5%). Penicillin 4 (4.8%) and tetracycline 6 (7.1%) were the least susceptible. The findings from this study emphasize the need for continual surveillance of MRSA and of antibiotic resistance in general.

Introduction

Methicillin-resistant *Staphylococcus aureus*

(MRSA) is considered a major nosocomial and community acquired pathogen throughout the world. MRSA is implicated in serious clinical conditions such as bacteremia, pneumonia, and intra-abdominal infection.¹ *Staphylococcus aureus* strains resistant to methicillin have become a well-known etiological agent in a wide variety of infections. These infections have become a common problem in hospitals and in the community, and have been associated with prolonged hospital stay and increased hospital costs.

Moreover, few therapeutic options are available to treat infected patients.^{1,2}

One of the earliest reports on MRSA in Africa was by Scragg *et al.*³ Subsequently, MRSA was reported from the mid '80s to the 1990s in a number of African countries including Ethiopia,⁴ Kenya,⁵ Nigeria,^{6,7} Senegal,⁸ South Africa and Sudan.⁹⁻¹² Resistance to methicillin (and to all β -lactam antibiotics) in *Staphylococcus aureus* is primarily associated with the acquisition of the *mecA* gene coding for the penicillin-binding protein 2a (PBP 2a), involved in bacterial cell wall synthesis.² A distinctive feature of methicillin resistance is its heterogeneous nature,^{13,14} with the level of resistance varying according to the culture conditions and β -lactam antibiotic being used. The majority of cells in heterogeneous strains (typically 99.9% or more) are susceptible to low concentrations of β -lactam antibiotics, *e.g.* 1 to 5 μ g/mL of methicillin.¹⁴

The detection of methicillin resistance, however, is complicated by the fact that its phenotypic expression in many strains is heterogeneous.¹⁵ This has resulted in the development of various laboratory techniques to enhance the expression of this resistance *in vitro*.^{3,4} Examples of these methods are slide latex agglutination test, rapid solid-phase immunoassay, and various disc diffusion methods. *mecA* gene detection tests based on polymerase chain reaction (PCR) or DNA hybridization have proved to be more specific and more sensitive than conventional tests, particularly in very heterogeneous strains.⁵ Little work has been done on MRSA in Ghana. The objective of this study was to determine the prevalence and antibiogram of MRSA in Accra, Ghana.

Materials and Methods

Clinical specimen

Two hundred and fifty (250) *Staphylococcus aureus* isolates from routine microbiological specimens were collected from five hospitals in Accra. Categories of patients included those hospitalized with suspected clinical infections (in-patients) and those attending the outpa-

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tients' department for the first time without hospitalization (out-patients). Only one isolate was recovered from each patient, presenting with a particular case. The *S. aureus* isolates were obtained from cultures of different specimens including wounds, sputum, blood, aspirates, urine, and cerebrospinal fluids. These isolates were collected and sub-cultured on slopes of Mueller Hinton agar for storage.

Patient bio-data

Full patient bio-data such as age, sex, and types of infection was obtained from request forms.

Culture

Suspected colonies of *S. aureus* from primary culture plates of Blood agar, Chocolate agar and MacConkey agar were confirmed as *S. aureus*, by Gram Reaction, positive catalase, Tube coagulase and Deoxyribonucleases (DNase) test. MRSA screening assay was used to screen for Methicillin resistance. The MRSA- screen assay is a 5-minutes slide latex agglutination test based on detection of PBP2a. The method involves extraction of PBP2a from suspensions of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. Inocula for susceptibility testing

were made using suspensions from over night-cultures of MRSA on Müller-Hinton agar. An inoculum equivalent to 0.5 McFarland (10^8 CFU/mL) turbidity standard was used for each test. Disc diffusion method (Kirby-Bauer method) was used to determine the susceptibility of the MRSA, to commonly used antimicrobial agents. These included: Penicillin (1 ug), Ampicillin (10 ug), Flucloxacillin (5 ug), Erythromycin (5 ug), Tetracycline (10 ug), Cotrimoxazole (25 ug), Cefuroxime (30 ug), Gentamicin (10 ug). The inoculum was compared to a 0.5 McFarland standard and the control was MRSA (ATCC 6571).

Results

From Table 1, a total number of 84 isolates from 250 *S. aureus* isolates tested positive for MRSA. Out of this, 87 MRSA wound specimen produced the highest (46) of MRSA representing 54.7% of total MRSA detected. This is followed by isolates specimen from swabs representing 21.4% of MRSA isolated. Blood specimen is the third highest (14.3%) source of MRSA. This is followed by Aspirates (3.6%), Sputum (2.4%) and Cerebrospinal fluid (2.4%). Urine specimen was the least only with 1 specimen representing (1.2%) tested positive for MRSA.

Figure 1 shows the percentage of MRSA and susceptible *Staphylococcus aureus* (MSSA). The prevalence of MRSA from this study is therefore 33.6%.

Distribution of methicillin-resistant *Staphylococcus aureus* isolates

Table 2 shows the distribution of 84 MRSA isolates recovered in terms of out-patients and in-patients. In patients had the highest carriage of MRSA representing 69%, while out patients had a total of 26 isolates representing 31%. No MRSA (0%) was detected from sputum, aspirates, cerebrospinal fluids and urine specimen recovered from out patients.

Discussion

Undoubtedly the epidemiology of MRSA has continued to evolve since its first appearance more than three decades ago. Initially there were sporadic reports of methicillin resistance amongst nosocomial *S. aureus* isolates. But later MRSA became a well established hospital acquired pathogen with few reports of community acquired isolates. Recent studies report of increases of prevalence of MRSA. In this present study (Figure 1) 33.6% of *S. aureus* isolates

Figure 1. Percentage of methicillin-resistant *Staphylococcus aureus* isolated from clinical specimen.

Figure 2. Multidrug resistance of methicillin-resistant *Staphylococcus aureus*. Multi drug resistance of methicillin-resistant *Staphylococcus aureus*. Generally, out of the 84 MRSA isolates, 25% were resistant to more than five antibiotics used, followed by 22.6%, 21.4%, 14.3%, 8.3% and 4.8% for six antibiotics, seven antibiotics, four antibiotics, three antibiotics and eight antibiotics respectively. Also 2.4% of MRSA were resistant to two antibiotics. While 1.3% were resistant to one antibiotic. In general 96.3% of the isolates were resistant to three or more antibiotics.

were found to be resistant to methicillin, whereas in the previous studies done in Accra, prevalence was found to be 22% in 2000 and 31.5% in 2002 (see Safo-Kantaka A, 2000. The studies of Methicillin resistant *Staphylococcus aureus* (MRSA) in Korle-Bu Teaching Hospital. *Personal communication*). This implies that the incidence of MRSA keeps changing every year and is on a rise as compared to the last few years.

The MRSA screen assay detected 84 of the 250 *S. aureus* isolates as MRSA (Table 1). No agglutination occurred when the 250 isolates were tested with the negative-control latex. The MRSA PBP2a latex agglutination screen assay is latex particles sensitized with anti-clonal antibody against PBP2a and react specifically with MRSA to cause agglutination. It is reported to have 97.6% sensitivity and it distinguishes between very low level MRSA from MSSA.^{16,17} Bowers et al., reported MRSA-latex agglutination test as a reliable and rapid detection test from both broth pure culture and selective media as well as being a reliable alternative to mec A PCR for the definite diagnosis of MRSA.¹⁸ Interestingly, Atoum et al. reported strains of negative mecA being methicillin resistant and positive mecA being methicillin sensitive. These observations are explained as being due to non functional mecA gene or non active PBP2a protein. Consequently, they recommended the use of a combination of both molecular and microbiological methods for detection of MRSA.¹⁹ However PBP2a latex agglutination has demonstrated 100% agreement for both *mecA*- positive and negative strains.²⁰

It is well established that the major mechanism of resistance to methicillin and other antistaphylococcal penicillins in *S. aureus* involves the production of an additional membrane penicillin-binding protein. In addition to the PBP2a production, most methicillin-resistant *S. aureus* also produce β -lactamases.²¹ There is however, limited information about the relationship between β -lactamase(s) of MRSA and the four β -lactamases (types A, B, C and D) produce by methicillin susceptible strains of *S. aureus*.

Most of the MRSA strains (34.8%) in this study came from wound and skin swabs (Table 2). This is similar to the study by Felten *et al*, in which it was reported that MSSA from skin lesions probably acquired the *mecA* gene by horizontal transfer from other skin *Staphylococcus species*.¹

MRSA is generally known to be resistant to β -lactam antibiotics (Figure 2). However penicillin, ampicillin and flucloxacillin (all β -lactam antibiotics) were included in the study to ascertain their antibiotic susceptibility patterns. From Table 3 penicillin had the highest resistance of 92.2% followed by ampicillin 84.5% and flucloxacillin 71.4%. These findings

Table 1. Isolates of methicillin-resistant *Staphylococcus aureus* from different clinical specimen.

Clinical Specimen	No. of <i>S. aureus</i> n=250	No. of MRSA n=84	% MRSA
Wound	87	46	54.7
Swabs	55	18	21.4
Blood	27	12	14.3
Aspirates	30	3	3.6
Sputum	25	2	2.4
Cerebrospinal fluid	17	2	2.4
Urine	9	1	1.2
Total	250	84	100

A total number of 84 isolates from 250 *S. aureus* isolates tested positive for methicillin-resistant *Staphylococcus aureus* (MRSA). Out of this, 87 MRSA wound specimen produced the highest (46) of MRSA representing 54.7 % of total MRSA detected. This is followed by specimen from swabs representing 21.4 % of MRSA isolated. Blood specimen is the third highest (14.3 %) source of MRSA. This is followed by Aspirates (3.6%), Sputum (2.4%) and Cerebrospinal fluid (2.4%). Urine specimen was the least only with 1 specimen representing (1.2 %) tested positive for MRSA.

Table 2. Distribution of methicillin-resistant *Staphylococcus aureus* isolates (outpatients and inpatient).

Clinical Specimen	No. of MRSA n=(84)	Outpatients No. (%)	Inpatients No. (%)
Wound	46	15 (32.6)	31 (67.4)
Sputum	2	0 (0)	2 (100)
Aspirate	3	0 (0)	3 (100)
Blood	12	4 (33.3)	8 (66.7)
Swabs	18	7 (38.9)	11 (61.1)
Cerebrospinal fluids	2	0 (0)	2 (100)
Urine	1	0 (0)	1 (100)
Total	84	26 (31.0)	58 (69.0)

Distribution of 84 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from outpatients and inpatients. In patients had the highest carriage of MRSA representing 69%, while out patients had a total of 26 isolates representing 31%.

Table 3. Antibiotic percentage susceptibility profile of methicillin-resistant *Staphylococcus aureus* (n=84).

ANTIBIOTICS	Sensitive No. (%)	Resistant No. (%)
Penicillin (1 ug)	4 (4.8)	80 (95.2)
Ampicillin (10 ug)	13 (15.5)	71 (84.5)
Flucloxacillin (5 ug)	24 (28.6)	60 (71.4)
Erythromycin (5 ug)	63 (75.0)	21 (25.0)
Tetracycline (10 ug)	6 (7.1)	78 (92.8)
Cotrimoxazole (25 ug)	35 (41.7)	49 (58.3)
Cefuroxime (30 ug)	33 (39.3)	51 (60.7)
Gentamicin (10 ug)	46 (54.7)	38 (45.2)

Resistant and sensitivity patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) to various antibiotics. Penicillin recorded the highest resistance (80) to MRSA, while erythromycin showed least (21).

agreed with the work done by Taiwo *et al.*²² The difference in the resistance of these β -lactam antibiotics is explained by the fact that PBP 2a-binding affinities differ among the various β -lactam antibiotics; however, there is cross-resistance to this general class of antibiotics because most, if not all, of the currently available agents bind too poorly to be active at clinically relevant concentrations.²³

MRSA isolates also showed resistance as follows: tetracycline 92.8%, and cotrimoxazole 58.3% (Table 3). High resistance of MRSA to gentamicin has also been reported.²⁴ However, in this study only 45.2% resistance to gentamicin was found. Furthermore, in another study, conducted among the aminoglycosides, findings revealed that susceptibility was maximum to netilmicin 52.5% and minimum to gentamicin 10.3% (showing that MRSA was 90% resistant to gentamicin).²⁵ However, because of various reasons, netilmicin cannot be recommended for empirical treatment of MRSA associated infections.²⁶ Lyon *et al.* have also shown that the majority of MRSA strains are also resistant to erythromycin, clindamycin, and tetracycline.²⁷

Conclusions and Recommendations

First, the findings of this research have shown that there is an increasing prevalence rate of MRSA in Accra, Ghana. To reduce the prevalence of MRSA, regular surveillance of hospital associated infections, monitoring of antibiotic sensitivity patterns of MRSA and formulation of definite antibiotic policy may be helpful. There is therefore the need for health policy planners to be in the forefront in the development of comprehensive programs, policies and strategic measures as well as the development of research and surveillance units. It is recommended that molecular characterization is done in further studies.

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