Methicillin-Resistant \textit{Staphylococcus aureus} (MRSA) Surveillance in Diabetic Foot Clinic (DFC)


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AIM: To investigate the prevalence of methicillin-resistant \textit{S. aureus} (MRSA) among infected/colonized foot ulcer, nasal carriage and hospital environment in diabetic foot clinic. METHODS: During 3 months duration among 64 selected diabetic patients, \textit{S. aureus} were identified, then Methicillin resistance detection was done by oxacillin disk diffusion tests, and confirmed by detection of PBP2a production by screen latex agglutination test. All MRSA isolates were tested for their antibiotic susceptibilities by the NCCLS agar disk diffusion method. RESULTS: Infection/colonization rate among 64 selected diabetic patients was 41% vs. 17%. Nasal carriage of MRSA was detected among 21 patients (34%) and one isolates from 6 studied HCW (16%). Only 4 MRSA isolates (13%) were isolated from the desk of the DFC at the end of the clinical session. Multiple resistance to non-ß-lactam antimicrobials (erythromycin, clindamycin, gentamicin and tetracycline), was also highly prevalent among the infected and nasal colonization of MRSA isolates by 30% and 5% respectively. CONCLUSION: This study underscores the need for hospital clinicians to be aware of the common bacterial isolates in their unit and their usual antibiotic susceptibility. This is imperative in order to make rational decisions for the prudent use of antibiotics, particularly for empirical therapy.

INTRODUCTION

Foot infection with MRSA has been associated with longer hospital stay, greater costs and greater mortality than infection with MSSA as reported by Lipsky et al., thus, prevention of MRSA colonization/infection is warranted.

Various studies have demonstrated an increase in the incidence of MRSA in patients with diabetic foot infection (DFI), these reports demonstrate MRSA involvement in 20% to 30% of DFI. This rate has significantly increase over the past 5 years, more troublesome is the fact that the presence of MRSA appears to significantly increase the risk of lower extremity amputation.

Several studies have identified specific modes of transmission via hospital reservoirs such as the anterior nares of the patient, inanimate objects within close proximity of the patient, and direct contamination from health care providers. Screening for carriage of MRSA is fundamental to modern-day nosocomial infection control, both for epidemiologic investigation and day-to-day decisions on barrier isolation, also knowledge of the local predominant pathogens helps in the selection of empirical antibiotic treatment, and the frequent isolation of MRSA from infected foot ulcers must be taken into account when treatment with antibiotics is prescribed.

Aim of the current research is to investigate the prevalence of methicillin-resistant \textit{S. aureus} (MRSA) among infected/colonized foot ulcer, nasal carriage and hospital environment in diabetic foot clinic.

PATIENTS AND METHODS

Settings:

Diabetic foot clinic (DFC) is newly constructed unit in Mansoura Specialized Medical Hospital with 7 health care workers (HCW) weekly inspect diabetic patient for foot problem mainly diabetic foot ulcerations, the infection control team regularly inspect the policies at regular intervals.

Subjects:

For a 3 months duration started from March-May 2006, a total of 64 consecutive patients attending DFC were included in the study, they were selected on the base of non antibiotic usage in the previous one month. Ulcers were also classified according to the Wagner classification system. Infection was diagnosed according to the criteria proposed by the international consensus on the diabetic foot. Non of the studied patients had infections at other body sites, and all patients had single ulcers only.
Data recorded included age, diabetes duration, type of diabetes, treatment, and most recent (within the last 6 months) glycosylated haemoglobin (HbA1c) level. HbA1c was measured. Peripheral sensory neuropathy was considered present if three or more sensory modalities were absent. Peripheral vascular disease (PVD) was diagnosed if both foot pulses (dorsalis pedis and posterior tibialis) were absent on palpation from the ulcer-affected limb. Neuroischaemic ulceration was diagnosed if criteria for both PVD and sensory neuropathy were met.

Sample collection

Ulcer sampling

The ulcer site and size were examined with the patient laying supine on an examination table. Superficial dead tissue or eschar was removed with sterile scissors and a scalpel blade. After local debridement of devitalised tissue, the wound was cleaned with sterile saline, samples of devitalised tissue were obtained from the depth of the wound, taking aseptic precautions.

Nasal sampling:

Nasal samples were obtained with sterile cotton-wool swabs from patients and health care workers (HCW), both the left and right anterior nares were swabbed by rubbing the swab 4 times around the inside of each nostril while applying an even pressure and rotating the swab without interruption.

Environmental sampling

Sampling surfaces and shared items in the Diabetic foot unit were done, at each site, an area of approximately 10 cm² in size was swabbed by rotating a sterile cotton swab, which had first been immersed in phosphate-buffered saline, in 3 directions as reported by Kazakova, et al. All environmental sampling was performed at the end of the clinical session.

Laboratory investigation

Bacteriological identification of S. aureus:

The clinical specimens and carrier screening samples were inoculated on to blood agar plates, and incubated at 37°C for 24 hours. Standard microbiological methods for the identification of S. aureus isolates were applied according to standard laboratory procedures. No duplicate isolates from a single patient were included.

For environmental samples after incubation in brain-heart infusion broth at 37°C overnight, then the bacterial suspension was tested for methicillin resistance.

Methicillin resistance detection

1) Oxacillin disk diffusion tests

The entire surface of the MHA plate was covered with the required inoculum, and the plate was air dried for 15 min before the disks were laid on the surface and incubation was performed for 18 h at the required temperature. Oxacillin resistance was determined with 1µg disks according to the NCCLS.

(2) MRSA-Screen latex agglutination test

Confirmation of Oxacillin resistant was done by detection of PBP2a production by Screen latex agglutination (Oxoid, Inc., Ogdensburg, N.Y.), which is based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a, it was performed according to the manufacturer instructions as reported by Felten et al.

Antimicrobial susceptibility testing

All MRSA isolates were tested for their susceptibilities by the NCCLS agar disk diffusion method on Mueller-Hinton agar using the disc diffusion technique as outlined by the National Committee for Clinical Laboratory Standards. The following drugs and concentrations (in brackets) were used to determine the antibiogram of the strains, first line antibiotics: Penicillin G (10U), ampicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg), cephradine (30 µg), cefuroxime (30 µg), gentamicin (10 µg), tetracycline (30 µg), and trimethoprim–sulphamethoxazole ((1.25/23.75 µg). The strains resistant to the first line were tested against second line antibiotics: vancomycin (30 µg), amoxicillin/k clavulanate (20/10 µg), fusidic acid (10 µg), rifampicin (5 µg), and ciprofloxacin (5 µg).

RESULTS

Patient characteristics

These are shown in Table 1, the 64 patients were 43 men and 21 women most had
type 2 diabetes. The mean age was 45 years. The majority of ulcers were neuropathic or neuroischaemic (86%).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean with range)</td>
<td>45 (25–88)</td>
</tr>
<tr>
<td>Duration of diabetes (mean with range)</td>
<td>14 (1–48)</td>
</tr>
<tr>
<td>Male : female ratio</td>
<td>43:21 (61%:29%)</td>
</tr>
<tr>
<td>Type 1: type 2 ratio</td>
<td>10:54 (15%:84%)</td>
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</tbody>
</table>

Table: Treatment

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<tr>
<th>Treatment</th>
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<tbody>
<tr>
<td>Diet</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Drugs</td>
<td>28 (43.7%)</td>
</tr>
<tr>
<td>Insulin</td>
<td>34 (53.1%)</td>
</tr>
</tbody>
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| HbA1c (mean with range) | 8.5% (4.8–13.6%) |

Table: Type of ulcer

<table>
<thead>
<tr>
<th>Type of ulcer</th>
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<tbody>
<tr>
<td>Neuropathic</td>
<td>34 (53%)</td>
</tr>
<tr>
<td>Ischaemic</td>
<td>10 (15%)</td>
</tr>
<tr>
<td>Neuroischaemic</td>
<td>20 (31%)</td>
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Bacteriological results:

All MRSA isolates with oxacillin resistance had positive results by Screen latex agglutination test.

MRSA prevalence in ulcer samples: Out of the 64 foot ulcers, positive bacterial culture were detected from 48 sample (75%). 22 isolates were identified as S.aureus (45%). Twenty MRSA isolates were detected out of 22 S.aureus (90%).

Foot ulcers were classified as 36 infected while 28 were uninfected. MRSA isolates were more common among patients with infected foot ulcers than among those with uninfected foot ulcers 15 and 5 and (41% vs. 17%, respectively).

Patients nasal MRSA carriage prevalence:

Twenty-one patients (32%) were identified as positive nasal carriage of MRSA of the 64 studied patients.

Among 36 infected foot ulcer: out of 15 patients with wound infected with MRSA, nasal MRSA carriage was detected among 11 patients (73%) while only 4 carrier (19%) were diagnosed among 21 patients with negative bacteriological result.

But among the 28 uninfected ulcers, out of 5 cases with wound infected with MRSA, nasal MRSA carriage was diagnosed among 4 patients (80%), while only 2 MRSA carrier (8%) were diagnosed among 23 patients with negative bacteriological result.

Health care workers nasal MRSA carriage prevalence: Among the six studied HCW
only one member (nurse) had positive nasal MRSA carriage (16%), she was persistent carriers (always culture positive).

**Environmental samples:**
One hundred and twenty specimens were taken from the inanimate environment of the DFU (20 specimen every screening program). Only 4 MRSA isolates (13%) were isolated from the desk of the DFU at the end of the clinical session. The CFU of all the environmental samples with positive MRSA result exceeded the upper detection limit of the $1 \times 10^2$ CFU/plate.

**Antimicrobial susceptibility testing**
A total of 20 isolates were identified from ulcer site (15 and 5 from infected and uninfected ulcers respectively), 21 isolates as 20 nasal strains from the patients and one from HCW.

From the infected wound isolates ($n = 15$) and nasal carrier ($n = 22$) isolates of MRSA the highest resistance was found to penicillin G, ampicillin and cephadine (100%), gentamycin (98% vs 96%), cefuroxime (96% versus 90%), followed by erythromycin and clindamycin (92% vs 89%) for both of them, trimethoprim–sulphamethoxazole (92% vs 87%), amoxicillin/k clavulanate (82% for both strains). The least resistance was shown to rifampin (6% vs 1%) followed by fusidic acid (20% vs 12%), ciprofloxacin (40% vs 12%), and tetracycline (75% vs 33%) respectively, all MRSA isolates were susceptible to vancomycin as shown in Fig 1.

Multiple resistance to non-β-lactam antimicrobials (erythromycin, clindamycin, gentamicin and tetracycline), was also highly prevalent among the infected and nasal colonization of MRSA isolates by 30% and 5% respectively.

![Antibiotic susceptibility patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolates](image)

Similar susceptibility pattern was detected between 4 environmental isolates and the health care worker isolate and also 4 infected foot ulcer isolates (data not shown). Furthermore, the antibiotic susceptibilities of the 9 clinical isolates from each patient had the same pattern with their nasal carriage isolates (data not shown).
DISCUSSION

In the current study, all MRSA isolates by the oxacillin disk-diffusion test had positive results by screen latex agglutination test, which has accuracy for detection of PBP 2a approaches the accuracy of PCR and is more accurate than any susceptibility testing method used alone for the detection of MRSA as reported by Sakoulas et al. 17.

The prevalence and antibiotic susceptibility patterns of MRSA isolates obtained from diabetic foot ulcer, nasal samples of the patients and health care workers were determined. Twenty strains were isolated from infected and uninfected ulcer (15 & 5 respectively), 21 MRSA strains as nasal carriage from the patients and only one from the health care worker.

The prevalence of MRSA among diabetic patients was by 41% vs. 17% respectively among infected and uninfected foot ulcers, which is less than a recent report in Greece (61.1% vs. 12.5%, respectively), their high prevalence of MRSA in patients with foot ulcers may reflect the increased prevalence of MRSA in their community.

In the current study, we detected that the rate of nasal carriage among the studied patients in both of infected and uninfected ulcer was higher in comparison to Saxena et al. 18 among the similar studied group of patients with type-II diabetes. Those results confirmed the importance of screening nasal carriage of MRSA among the diabetic patients with foot problem as MRSA colonization of nares increases the risk for MRSA infection as reported by Stanaway et al. 19.

This high prevalence confirms that patients admitted to our DFU were at particularly high risk for MRSA acquisition, probably because they had a combination of several known risk factors, such as diabetes mellitus, surgical intervention, close contact with hospital personnel, or exposure to a healthcare setting (particularly if repeated) within six months 20,21.

Higher prevalence of HCW carriage of MRSA was reported by Opal et al. 22 but we found lower prevalence (16%) in our clinic in accordance to Koffi et al. 23, as they explained that this variation can be caused by the geographic distribution, hospital ward, studied population with endemic or epidemic MRSA infection, rotation of the HCW among different hospital wards and also the number of HCW in the clinic.

Many of the studies implicating the environment in the transmission of MRSA have been conducted during outbreaks, which were not been brought under control until the environment has been thoroughly cleaned 24, but in the current study 13% of environmental screening yielded MRSA isolates from a desk in the clinic after application of infection control measures, being more frequently touched by hands of the HCW.

So, the desk may play a role in the transmission of MRSA by transferring the MRSA via the movement of dust in air currents to surfaces that are touched more frequently, also it was reported by Hardy et al. 25 that when colonized patients are in the ward, MRSA strains from patients may be found in the environment some distance from the colonized.

The important striking point in this research is the antibiotic sensitivity profiles of the isolated strains, as all the carrier strains were resistant to penicillin and most of them were resistant to ampicillin. This is in contrast to other studies where higher susceptibility rate was observed among the strains obtained from carrier screening samples 25. This might be due to the fact that at present time these agents are tremendously used in the treatment of general infections in our locality.

Also, for nasal colonization isolates the rate of antibiotic sensitivity varied in our community with that reported by Lu et al. 20. Recent receipt of medical services was the major factor associated with MRSA colonization as well as the high level of multiple-drug resistance in MRSA nasal isolates as reported by Dang et al. 5.

These findings are indicative of the presence of strong selective pressure from antimicrobial use in the community.

Another striking point in the research that we detected high degree of resistance among the MRSA isolated from infected ulcer against trimethoprim–sulphamethoxazole, amoxycillin–clavulanic acid, cephradine and...
The result of our research is very important as those antibiotics commonly prescribed on an outpatient basis.

The degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and vancomycin was the only antibiotic found to give uniform sensitivity (100%). Our study is a preamble to enable epidemiologists to understand the nature of MRSA isolates.

The rate of multiple resistance to at least four non-ß-lactam antimicrobials among the MRSA in this outpatient studied group was unusually high for community-MRSA, as we detected that among the infected and nasal colonization of MRSA isolates 30% and 5% respectively were multiple resistance. These data are consistent with a recent report documenting high prevalence of multiple resistance in community MRSA from Taiwan by Lu et al. and may reflect high antimicrobial usage in the community.

By investigating the phenotypic pattern of MRSA isolates by antibiotic susceptibility testing, we detected that 4 MRSA isolates from the environment and the 3 air isolates had the same susceptibility patterns as the isolates taken from the HCW isolate and 4 infected patients foot ulcer isolates (data not shown).

Four isolates from the patient had a susceptibility pattern consistent with that of HCW isolates. This observation is most likely explained by either acquisition during the previous documented clinic visit, acquisition during another, unidentified healthcare encounter, or community acquisition of a MRSA strain that originated in a healthcare facility.

Furthermore, the antibiotic susceptibilities of the 9 clinical isolates from each patient had the same pattern with his nasal carriage isolates (data not shown), so we recommend more education program for the diabetic patients to prevent cross infection and trying to eradicate MRSA carrier from them.

Further use of molecular studies to monitor the epidemiology of MRSA in our unit is highly recommended, as a problem of our study that we can't make genotyping testing to demonstrated similarity of MRSA isolates between nasal and ulcer strains from diabetic foot ulcer patients, nor with patients, HCW and the environmental strains.

An important question is whether eradication of nasal MRSA from diabetic patients may reduce foot ulcer infection with MRSA, also further study is required.

But on the other hand we recommend that eradication of MRSA carriage among HCW is very important as Muto et al., reported that although eradicating carriage of MRSA is very difficult and should not been attempted routinely but should be considered for specific situations, such as HCW who has been epidemiologically linked to an outbreak, a patient for whom MRSA colonization poses a particular risk of infection or of transmission to others (similar group of our patients).

In conclusion: regional differences in the prevalence of MRSA and MRSA antibiotic sensitivity should be considered in order to achieve better control of MRSA colonization or infection in DFC, also knowledge of the local predominant pathogens helps in the selection of empirical antibiotic treatment, the frequent isolation of MRSA from infected foot ulcers must be taken into account when treatment with antibiotics is prescribed.

REFERENCE


The study aimed to investigate the incidence of Entamoeba coli, Isolates of E. coli were obtained from the patients' stool samples and cultured on MacConkey's agar. The results showed that the incidence of E. coli was 14% among the patients with diabetes mellitus. The study also found that the incidence of E. coli in the patients with diabetes mellitus was significantly higher than in the control group (71%). The study concluded that the use of antimicrobial agents in the treatment of diabetes mellitus is warranted to prevent bacterial infections.