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Antibiotics 2019 : Multi-drug resistance of MRSA isolated strains from healthcare, community and the distribution of fusidic acid MIC and zone of inhibition - Said E Wareg - University of Tripoli

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The evolution of resistance to antibiotics is one of the most significant problems in Modern medicine, posing serious threats to human and animal health. Multidrug-resistant organisms (MDRO, S), including MRSA, vancomycin-resistant Enterococci (VRE) and certain Gram-negative bacilli have important infection control complications. From a previous study, an agar susceptibility testing was used to test all isolates against vancomycin, chloramphenicol, gentamicin, fusidic acid, erythromycin, streptomycin, Ciprofloxacin, cefotaxime and clindamycin.

MRSA was detected using cefoxitin (30µg) disc and antibiotic susceptibility pattern was determined using the Kirby and Bauer disc diffusion susceptibility testing method and confirmed for fusidic acid and vancomycin by determination of minimum inhibitory concentration. The isolated MRSA strains showed multiple drug resistance pattern as 42% for IPHA-MRSA, 34% for OPHA-MRSA and 23% for CC-MRSA. The distribution of strains of IPHA, OPHA and CC-MRSA compared with the MIC and zone size of fusidic acid showed that the highest number of isolates were distributed about the highest MIC values for IPHA-MRSA(16 , 8 , 4mg/L) ,OPH-MRSA (8, 4,2mg/L) and CC-MRSA(8,4mg/L). These results showed high multi-drug resistance for all MRSA isolated strains (Fig.1, Fig.2, Fig.3). Interpretative zone of inhibition for fusidic acid and vancomycin was based on the British Society for Antimicrobial Chemotherapy (BSAC) (Anon-2010/2013) guidelines. Standard international interpretation criteria for zone size for fusidic acid should be addressed.

Keywords: MRSA: Methicillin-Resistant *S. aureus*, *IPHA*-MRSA: In-patient health associated MRSA: OPHA-MRSA: Out-patient health associated MRSA; CC-MRSA: Community carried MRSA.

Introduction

Multi-drug resistance:

The evolution of resistance to antibiotics is one of the most significant problems in modern medicine, posing serious threats to human and animal health. The early work on the use of antibiotics to treat bacterial infections gave much hope that infectious diseases were no longer a problem, especially in the human field. However, as their use, indeed overuse or abuse progressed, resistance which was transferable between different strains and species of bacteria emerged.

Multidrug-resistant organisms (MDROs), including methicillin-resistant *S. aureus* (MRSA), Vancomycin-Resistant *Enterococci* (VRE) and certain Gram-negative *bacilli* have important infection control implications that either have not been addressed or received only limited consideration (Jom et al. 1998). In a routine ward environment study by Tan et al. 2013 clinical isolates recovered from the hands of healthcare professionals showed that MDROs were recovered from 79% of sampled surfaces, predominantly MRSA (74% of all tested surfaces), *Acinetobacter baumannii* (29%), VRE (2%) and *Klebsiella pnuemoniae* (1%). Although the names of certain MDROs describe resistance to only one agent (e.g. MRSA-VRE), these pathogens are frequently resistant to most available antimicrobial agents. MRSA and VRE, other β-lactamase producing organisms resistant to multiple classes of antimicrobial agents, are of particular concern (Mahgoub et al. 2002). Although antimicrobials such as linezolid, telavancin, daptomycin and oxazolidines are now available for treatment of MRSA and VRE infections, resistance to each new agent has already emerged in clinical isolate (Skov et al. 2012).

Materials and methods

Bacterial strains:

From a previous study in-patient health associated MRSA (IPHA-MRSA) isolates were isolated from 100/ 43% of clinical samples, out-patient health associated MRSA (OPHA-MRSA) were isolated from 62/ 37% and for community carried MRSA (CC-MRSA) strains isolated from 38/ 34% of clinical samples.

Antimicrobial susceptibility testing:

S. aureus ATCC25923 and isolates were inoculated into Nutrient Broth (Oxoid-64065) and incubated at 37°C for 18-24 h. The cultures were diluted with fresh NB to give a turbidity equivalent to 0.5 McFarland standard absorbance at 625nm (optical density of 0.08-0.13). Susceptibility tests were performed by the disc diffusion method of Bauer et al. (1966) with Mueller-Hinton agar (Oxoid-CM0337) supplemented with 20 g1-1 NaCl. Cefoxitin was used as an indicator of methicillin susceptibility. Zones of inhibition were measured after 18 and 24 h incubation at 35°C. S. aureus ATCC25923 (Biosafety level /BSL) was the control strain used in every run. Tests were performed in duplicate. Six discs were accommodated on a 13.5 cm plate for each run. Antibiotic discs used were vancomycin (VAN) 30 µg, chloramphenicol (CHL) 30 µg, gentamicin (GEN) 10 µg, fusidic acid (FUS) 10 µg, erythromycin (ERY) 15 µg, streptomycin (STR) 10 µg, cefoxitin (as an indicator of methicillin-resistance) (FOX)30 µg, cefotaxime (CTX) 30 µg,

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clindamycin (CLI) 2 µg and ciprofloxacin (CIP) 5 µg. The isolates were reported as sensitive, intermediate and resistant based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (Anon. 2013).Interpretative zone of inhibition for fusidic acid which is not stated in the CLSI guidelines were reported as resistant ≤ 29 mm and susceptible ≥30 mm according to the British Society for Antimicrobial Chemotherapy (BSAC) (Anon-2010/2013) guidelines. Vancomycin zone size interpretation was also on the bases of BSAC guidelines.

Minimum inhibitory concentration

Antibiotic stock solution:

Stock solution was prepared using the formula 100/P X V X C = W, where: P= potency given by the manufacturer (µg/mg), V= volume required (ml), C= final concentration of solution (multiplies of 1000) (mg/L), W= weight of antibiotic (mg) to be dissolved in volume V (ml). Working stock solutions were prepared as follows: 20 ml of water =10,000 mg/L stock solution. Further stock solution from initial 10,000 mg/l prepared by the addition of 1 ml of 10,000 mg/l solution+9 ml water = 1000mg/l and 100µL of 10,000mg/l solution + 9.9 ml diluents = 100mg/l.

Preparation of antibiotic dilution range:

Antibiotic dilutions in the range 0.25-128 mg/L were prepared by labeling 11 universal containers marked 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0 mg/L. Doubling dilutions were prepared as follows from the 10,000mg/l stock the following amounts (256µL, 128µL, 64µL, 32µL) were dispensed to containers labeled (128, 64, 32, 16) respectively. From1000mg/L stock, the following amounts (160µl, 80µL, 49µL) were dispensed to containers labelled (8, 4, 2) respectively and from100mg/L stock, the following amounts: (200µL, 100µl, 50µl) were dispensed to containers labelled (1, 0.5 and 0.25) respectively. No antibiotic was added to the bottle labelled 0 mg/l (antibiotic free growth control).

Materials and Methods

Preparation of agar dilution plates:

Twenty milliliters of cooled molten nutrient agar was added to each container(containing antibiotic dilution) and the medium was cooled to 50°C before adding to the antibiotic. Each container including the antibiotic–free control were mixed well before pouring into 90 mm petri- dishes. The agar mix for each concentration was poured in turn so agents were kept at 50°C for the minimum period of time, the agar was allowed to set and then the surface of the plates were dried for 10 min in a fan-assisted drying cabinet (without ultraviolet) or in a still incubator, the plates were stored at 4-8°C and protected from light until inoculated and used on the day of preparation.

Growth of test organism:

At least four morphologically similar colonies were touched with a sterile loop. The growth was transferred into nutrient broth and incubated with shaking at 35-37°C until the visible turbidity was adjusted to equal the 0.5 McFarland standard. Preparation of inoculum: Inoculum was adjusted to 10 cfu/spot and applied to the plates. The suspensions were used within 30 min of preparation. Inoculation; 1-2 µl of suspension was used on the surface of the agar and the inoculate were allowed to be absorbed into the agar before incubation. Incubation conditions: 35-37°C for 24h. Reading and interpretation: After incubation, all of the organisms were checked for growth on the antibiotic-free control plate. The MIC is defined as the lowest concentration of antibiotic at which there is no visible growth, the growth of one or two colonies or a fine film of growth was discarded. Quality control: The quality of test results was monitored by the use of control strain ATCC25923. Working cultures and control strains were stored on semi-solid nutrient media and sub-cultured frequently. MIC results were estimated as the mean of three experiment runs.

Results

Fusidic acid resistant strains

This study also revealed that all MRSA strains that were isolated from healthcare and the community facilities in Libya were resistant to fusidic acid when tested using British Society for Antimicrobial Chemotherapy (BSAC)-version (2010/2013) guidelines. Two main fusidic acid resistance mechanisms have been reported: resistance relates to mutations in fus A, the gene that encodes the ribosomal translocase and translation elongation factor EF-G (Nagaev et al. 2001). Fusidic acid has been circulating in Libyan healthcare and community facilities for many years and is still currently used as a topical treatment for skin and soft tissue infection. MIC of isolated MRSA strains against fusidic acid and vancomycin. MIC range for fusidic acid was ≥ 4-32 mg/L for 82-97% of IPHA, OPHA and CC isolates. The MIC test results for fusidic acid confirms high resistance rate for all isolates (i.e. IPHA, OPHA and CC isolates). The European Committee on Antimicrobial Susceptibility Testing (EUCAST)-version 3.1/2013 fixed the fusidic acid MIC as susceptible ≤ 1 and resistant > 1 mg/L which is the same breakpoint stated in the British Society for Antimicrobial and Chemotherapy (BSAC) version 9.1. March 2010/2013. MIC testing interpretation criteria was based on the CLSI 2013 guidelines both the Clinical Standards Institute (CLSI) and the united States Food and Drug Administration (FDA) have stated the break points for fusidic acid 2 mgl-1 and all the strains were resistant with three IPHA-MRSA and 4 OPHA-MRSA isolates showing a high level of resistance of ≥ 64 mgl-1, even in the out-patient isolates in contrast to the results of the disc diffusion assay.

Distribution of fusidic acid MIC values in comparison with the zone of inhibition and the number of MRSA strains. The distribution of MIC values for IPHA-MRSA, OPH-MRSA and CC-MRSA for each zone size figures 1, 2, 3 showed that the highest number of isolates were distributed about the highest MIC values for IPHA-MRSA (16, 8, 4mg/L) OPH-MRSA (8, 4, 2mg/L) and CC-MRSA (8, 4mg/L) respectively. These MIC and number of isolates were distributed in the vicinity of the zone size range of (11-15/16mm) for the three groups.

Discussion

Multi-drug resistance among isolated MRSA strains

The isolated strains of MRSA displayed full resistance to fusidic acid and multiple drug resistance (MDR) to 2-9 antibiotics for IP-MRSA, 2-7 antibiotics for OP-MRSA and 2-6 antibiotics for CC-MRSA. The most frequent MDR was resistance to fusidic acid, ciprofloxacin, streptomycin, cefotaxime and clindamycin. This observation is in agreement with previous studies in Libya (Buzaid et al. 2011). This study has shown that MRSA is prevalent with similar rates for IP-MRSA, OP-MRSA and CC-MRSA strains. Withdrawal of topical fusidic acid in dermatology department (Southern General Hospital/UK) led to a statistically significant fall in fusidic acid resistance rates (Wylie *et al.*, 2011). This might be just one of the measures that could be considered to minimize the spread of fusidic acid resistance in Libya and the implementation of large-scale prospective surveillance monitoring program. However, it's clear that there is not enough evidence for established standard international interpretation criteria for zone size for fusidic acid (Toma *et al.*, 1995).

Conclusion

Both HCA-MRSA and CA-MRSA had shown multiple resistances to a wide range of antimicrobials, a threat which causes much concern to clinicians and health professionals and is a great challenge to monitor resistance epidemiology on a regular basis. Gentamicin, ciprofloxacin and vancomycin can still be used to treat HCA-MRSA and CA-MRSA infections. The isolated strains of MRSA displayed full resistance to fusidic acid and multiple drug resistance. The highest number of isolates were distributed about the highest MIC values for IPHA-MRSA (16, 8, 4mg/L) OPHA-MRSA (8, 4, 2mg/L) and CC-MRSA (8, 4mg/L) respectively. Standard international interpretation criteria for zone size for fusidic acid should be addressed.

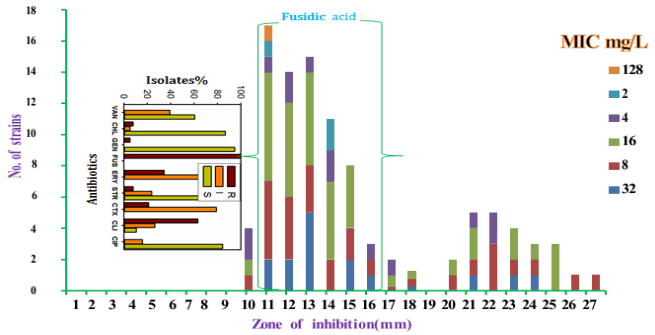

Figure 1. Distribution of the number of IPHA-MRSA strains compared with MIC and zone of inhibition for fusidic acid. Key. MIC= minimum inhibitory concentration. R= Resistance, I= Intermediate and S= Susceptible, VAN= vancomycin, CHL= chloramphenicol, GEN= gentamicin, FUS= fusidic acid ERY= erythromycin, STR= streptomycin, CTX= cefotaxime, CLI= clindamycin and CIP= ciprofloxacin.

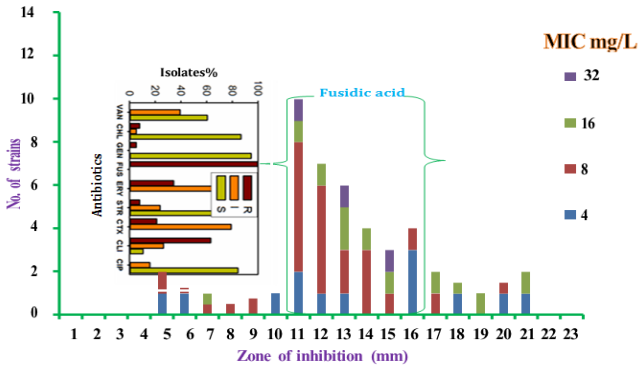

Figure 2. Distribution of the number of OPHA-MRSA strains compared with MIC and zone size for fusidic acid. Key: As in figure 1.

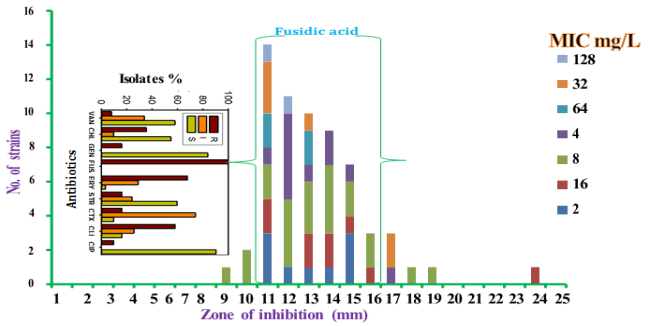

Figure 3. Distribution of the number of CC-MRSA strains compared with MIC and Zone size for fusidic acid. Key As in figure2.

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