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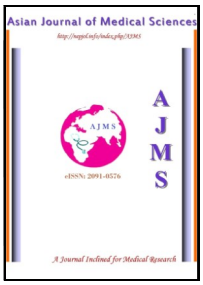


# Phenotypic Characterization and Antibiotics Combination Approach to Control the Methicillin-resistant *Staphylococcus aureus* (MRSA) Strains Isolated from the Hospital Derived Fomites

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## Abstract

**Objective:** The emergence of antibiotic resistant *Stphylococcus aureus* strains have resulted in significant treatment difficulties which have imposed a burden on health care systems and simultaneously intensifying the need for new antimicrobial agents. Therefore, we have designed the study to determine the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and their antimicrobial susceptibility pattern along with evaluation of antibiotic combination approach.

**Material & Methods:** Isolation and identification of MRSA was carried out using Mannitol salt agar followed by Baird-Parker Agar and Vojel-Johnson Agar media. Strains were tested for the sensitivity against five ß-lactum, two macrolide and one fluoroquinolone class of antibiotic. Further sensitivity was confirmed using 9 diverse- second and third generation antibiotics. MIC value of individual and mixture of antibiotics were measured using Mueller-Hinton broth.

**Results:** Total 10 stains of *S. aureus* were isolated and characterised using standard biochemical tests. They were identified using on-line identification tool- GIDEON (Global Infectious Disease and Epidemiology Network). Almost 50% strains were reported having variable reaction against at least one or more antibiotic. By the use of augmentin (30µg), cephotaxime (30µg), ceftriaxone (30µg), cefuroxime (30µg), ciprofloxacin (5µg), clindamycin (2µg), gentamycin (10µg), lincomycin (2µg) and tobramycin (10µg) further susceptibility tesing was carried out which reveals absolute resistant in 70% of strains, whereas, remeaning strains were seen having variable responses against five antibiotics. However, with comparison to standard antibiotic zone of inhibition, all strains were categorized as a resistant. An individual application of ampicillin, tetracycline and amikacin required higher concentration to inhibited all the stains while combination of two or three antibiotics were identified having low MIC value. Combination of tetracycline with sulbactam and cefoperazone was found best to contol MRSA.

**Conclusion:** Study highlights the wide spread presence of MRSA strains in fomites derived from hospital. These indicate the need of more precaution in clinic area to control the spread of the MRSA. Amalgamation of antibiotics is an effective remedy for MRSA infections and promising approach in developing country.

***Key Words:*** *Antibiotics combination; fomites; MRSA; Staphylococcus aureus*

## 1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen, causing a wide range of infections in healthy persons. MRSA has traditionally been considered a nosocomial pathogen, however recently MRSA emerged in the community[1] and more recently a specific sequence type of MRSA, emerged in farm animals and farmers, is referred to as livestock associated MRSA (LA-MRSA).[2, 3] These ubiquitously occurrences of MRSA infection are due to the multiple inanimate sources act as a potent reservoir of infection for acquiring *Staphylococcus* infections. Various inanimate objects have been evaluated for the presence of MRSA includes soap, soap dispensers and towels,[4, 5] moreover *S. aureus* transfers to surgical gowns[6] to cotton fabrics[7] and survive for days on various types of fabrics.[8, 9]

MRSA could be isolated from a variety of frequently touched surfaces including wiping cloths, dishtowels as well as badges, lanyards and coats worn by health care workers.[10-12] Contaminated environmental surfaces have been shown to play a significant role during outbreaks in long term care facilities. MRSA may be aerosolized in the droplet nuclei from a coughing resident and also survive in infectious dust generated from the patient's stuffs. Improper discard or storage of hospital fomites has a great concern towards the new infection of

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MRSA.[13] Present study includes the screening of multi-drug resistant *Staphylococcus aureus* from the discarded sputum samples, blood containing bandage and discarded surgical Cotton and Swab. Such hospital discarded waste is not only reservoir of MRSA but also a potential origin of many new reported multi drug resistant species other than MRSA.[14] This creates the need to investigate hospital waste during their storage, maintenance and transport. Currently, up to 60% of nosocomial infections of *S. aureus* are reported resistant to oxacillin[15] and more than 60% of the isolates are resistant to methicillin.[16]

The resistance of MRSA against various antimicrobials is globally increasing at alarming rate and treatment of MRSA infections has become more challenging since it is a major concern among health care professionals. The developments of new and effective antibiotics belonging to different classes are being aggressively pursued to combat this challenge. A glycopeptide antibiotic e.g. vancomycin is best example, currently used for the MRSA treatment. However, clinical isolates of MRSA with resistance to new classes of antibiotics have already been reported.[17-19] Therefore, there is a prompt requirement to develop inventive therapeutic agents or antibiotic substitutes that are active against MRSA. As alternative, current antibiotics to which MRSA strains are resistant may be revived as viable candidates in the treatment of MRSA when used in combination with other agents, offering a new dimension to curtail the emergence of *Staphylococcus aureus* strains.[20] So Such Synergistic interactions of multiple antibiotics are the effective approach to treat the MRSA infection.[21] Hence, proposed research focus on the control of MRSA with combine approach using new antibiotics with old one. Such strategy is also supported and promoted to fight against MRSA by numerous experts.[22] Study included the different concentrations of various antibiotics to evaluate the sensitivity of fomites isolated MRSA.

## 2. Material and Methods

### 2.1 Materials

All the chemicals, kits and microbiological media were purchased from Himedia Pvt. Ltd. Co., Mumbai, India.

### 2.2 Sample collection

Discarded fomites including towel, bandage, cotton and swab were collected in the sterile polythin bags from three hospitals of Gujarat state, India. Isolation of *Staphylococcus aureus* was done by enrichment method using Mannitol-Salt broth followed by the streaking with overnight old culture on same solid media, Mannitol-Salt Agar (MSA). The plates were incubated at 37°C temperature to allow the growth of the isolates. Further screening was done on Baird-Parker Agar (BPA) and Vojel -Johnson Agar (VJA) media.

### 2.3 Macroscopic examination

Colony characteristics were examined on MSA, BPA and VJA medium for phenotypic characterization of all the isolates. Hemolytic activity was detected on blood agar medium.

### 2.4 Antibiotics susceptibility

Susceptibility of isolates against eight different antimicrobial agents was determined by the agar disk diffusion method.[23] The following antibiotics at the indicated concentrations were tested against all the strains: penicillin (10U), ofloxacin (5µg), erythromycin (15µg), cefuroxyme (30µg), cephalexin (30µg), amoxicillin/clavulanic acid (20/10µg), amoxicillin (10µg) and azithromycin (15µg). To ensure the resistance of the MRSA strain other antibiotics were also tested e.g. augmentin (30µg), cephotaxime (30µg), ceftriaxone (30µg), cefuroxime (30µg), ciprofloxacin (5µg), clindamycin (2µg), gentamycin (10µg), lincomycin (2µg) and tobramycin (10µg).

### 2.5 Biochemical characterization and Identification

Innovative concept was used for the rapid identification of the isolates using online identification system of GIDEON available at public domain (http://web.gideononline.com/web/microbiology/identify_index.php-type=bacteria). Data of basics biochemical tests and colony characteristics were given as an input in on-line GIDEON tool. All the isolate strains were identified up to species level through the interpretation of GIDEON output data.

### 2.6 Antibiotics combination with MIC

Minimum inhibitory concentration (MIC) was performed ranging from 1 to 2048 µg/ml for individual antibiotics including ampicillin, tetracycline and amikacin and mixture of antibiotics using Mueller-Hinton broth by microdilution method for each strain. Three different kind of antibiotics were used in three different combination (1) sulbactam: cefoperazone=1:2 (2) ampicillin: salbactam: cefoperazone= 1:1:2 (3) Tetracycline: Sulbactam:Cefoperazone=1:1:2.

## 3. Results

### 3.1. Isolation of strains

Total 31 bacterial strains were isolated from hospital derived fomites. Out of them, 10 strains were selected for the study based on cultural characteristics. Five strains were isolated from surgical cotton and swab followed by three strains from blood containing bandage and two strains from Sputum containing towel.

**Table-1:** Antibiotics susceptibility response of strains

| Antibiotics (Concentration) | Inhibition zone diameter in millimeter by plate assay after 24 hours incubation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PtA* | PtB | PtC | PtD | PtE | RjA | RjB | RjC | RjD | RjE |
| Penicillin (10 U) | 0 | 0 | 0 | 0 | 0 | 3 | 9 | 4 | 7 | 0 |
| Ofloxacin (5 µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythromycin (15 µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cefuroxyme (30µg) | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 6 | 2 | 0 |
| Cephalexin (30µg) | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 5 | 0 | 0 |
| Amoxicillin/ clavulanic acid (20/10 µg) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Amoxicillin (10µg) | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 2 |
| Azithromycin (15µg) | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 1 | 0 |

* Strain

### 3.2. Macroscopic examination

Growth characteristics of strains on selective media including MSA, BPA and VJA shown different growth pattern respectively typical yellow colonies with yellow zones, black-shiny colonies surrounded by clear zone and black colony surrounded by yellow zones. All the strains were found Gram positive cocci. The microscopic and macroscopic characteristics were completely similar to the typical colony characters of *Staphylococcus aureus*. Hemolytic activity on blood agar plate was indicated by all the strains.

### 3.3. Antibiotics susceptibility

All the strains were found absolutely resistance towards ofloxacin and erythromycin, while the rest of the antibiotics were able to inhibit the growth with variable response against all the strains. Five strains PtA, PtB, PtC, PtD and PtE were indicated 100% resistance with lack of inhibition zone on plate against all antibiotics. However all strains were multi-drug resistance towards all antibiotics.

All the isolates from the TB hospital and civil hospital waste were reported as a multidrug resistance. But the isolates from the general hospital were seen having variable level of the resistance towards the all antibiotics which were included in study (Table-1). Further the testing of antimicrobial susceptibility pattern against nine antibiotics; three strains RjB, RjC and RjD were seen having variable response against three or more antibiotics. But these three strains were found resistant accordance to the comparison to standard zone of inhibition of all antibiotics and the rest were reported having total resistance against all other antibiotics (Table-2).

**Table-2: Antibiotic susceptibility pattern**

| Antibiotics (Concentration) | Inhibition zone diameter in millimeter by plate assay after 24 hours incubation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PtA | PtB | PtC | PtD | PtE | RjA | RjB | RjC | RjD | RjE |
| Augmentin (30µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cephotaxime (30µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| Ceftriaxone (30µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 3 | 0 |
| Cefuroxime (30µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciprofloxacin (5µg) | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 5 | 0 | 0 |
| Clindamycin (2µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamycin (10µg) | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 11 | 5 | 0 |
| Lincomycin (2µg) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tobramycin (10µg) | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 7 | 0 |

### 3.4. Biochemical characterization and identification

All the isolates indicated the positive result of various biochemical tests including nitrate reduction, Methyl Red test, Voges Proskauer test, catalase production and coagulase, where as $H_2S$ production, indol production, citrate utilization, urease activity, oxidase activity and starch hydrolysis indicated negative. The slightly variable response was recorded for the rest of biochemical tests. Identification was done using phenotypes including cultural characteristics, microscopic and biochemical properties through GIDEON (Table-3). All isolates having 100% probabilities of being *Staphylococcus aureus* according to GIDEON identification tool.

### 3.5. Antibiotics combination with MIC

For individual application of ampicillin, tetracycline and amikacin; the MIC range against all strains was recorded respectively 512 to 1024 µg/ml, 256 to 512 µg/ml and 128 to 512 µg/ml. The combined effect of sulbactam/ cefoperazone (128 to 256 µg/ml) and ampicillin / salbactam/

Cefoperazone antibiotics (128 to 256 µg/ml) have shown reduced MIC compare to individual antibiotics. However, there was no positive response of ampicillin/sulbactam / cefoperazone and MIC was similarly. MIC range for the combination of tetracycline /sulbactam/ cefoperazone was recorded at the 32 to 128 µg/ml. The stated figures of MIC was the range value of all the strains (Table-4).

**Table-3: Phenotype of *Staphylococcus aureus* used in the GIDEON**

| SN | Phenotypes | SN | Phenotypes | SN | Phenotypes |
|---|---|---|---|---|---|
| 1 | - Gram negative | 22 | - Glucose oxidizer | 43 | + Growth in 6.5% NaCl |
| 2 | + Gram positive | 23 | - Not identified by in-vitro tests | 44 | + Cephalothin-susceptible |
| 3 | + Coccus | 24 | + Beta hemolysis | 45 | - Colistin-Polymyxin susceptible |
| 4 | - Coccus - pairs or chains predominate | 25 | + Coagulase production | 46 | + Novobiocin-susceptible |
| 5 | + Coccus - clusters or groups predominate | 26 | - Motile | 47 | + Alkaline phosphatase |
| 6 | - Bacillus or coccobacillus | 27 | - Cholesterol needed for growth | 48 | - Pyrrolidonyl-ß-naphthylamide |
| 7 | - Branching filaments present | 28 | - X factor required | 49 | - Alpha-methyl-D-glucoside |
| 8 | - Spore formation | 29 | - V factor required | 50 | - L-Arabinose |
| 9 | - Acid fast | 30 | - Capnophilic | 51 | - Cellobiose |
| 10 | - Spirochete | 31 | - ONPG (beta galactosidase) | 52 | + Glycerol |
| 11 | - Curved bacilli | 32 | + Voges Proskauer | 53 | V Lactose |
| 12 | - Cell wall-deficient | 33 | + Nitrate to nitrite | 54 | + Maltose |
| 13 | - Aerobe | 34 | + DNase | 55 | + D-Mannitol |
| 14 | + Facultative | 35 | V Esculin hydrolysis | 56 | + D-Mannose |
| 15 | - Anaerobe | 36 | V Gelatin hydrolysis | 57 | - Melibiose |
| 16 | - Microaerophilic | 37 | V Lipase | 58 | - Raffinose |
| 17 | + Growth on ordinary blood agar | 38 | V Urea hydrolysis | 59 | - Salicin |
| 18 | - Growth on MacConkey agar | 39 | + Yellow pigment | 60 | - D-Sorbitol |
| 19 | - Oxidase | 40 | + Arginine dihydrolase | 61 | + Sucrose |
| 20 | + Catalase | 41 | - Ornithine decarboxylase | 62 | + Trehalose |
| 21 | + Glucose fermenter | 42 | + Growth at 42 degrees C | 63 | - D-Xylose |
| **(+) indicate positive result, (-) indicate negative result and (V) for variable response of strains** | | | | | |

**Table-4: MIC of individual and combination of antibiotics of all the strains**

| Sr. No. | Antimicrobial agent | MIC (µg/ml) range of all strains |
|---|---|---|
| 1 | Ampicillin | 512 to 1024 |
| 2 | Tetracycline | 256 to 512 |
| 3 | Amikacin | 128 to 512 |
| 4 | Sulbactam and Cefoperazone | 128 to 256 |
| 5 | Ampicillin with Sulbactam and Cefoperazone | 128 to 256 |
| 6 | Tetracycline with Sulbactam and Cefoperazone | 32 to 128 |

## 4. Discussion

Staphylococcal infections are significant clinical problem in medical practice. The prevalence of MRSA, however, varies markedly country wise. Even within country like India, MRSA strains were more common in southern India (30.94%) than the west (20.33%) and the north (18.88%) side of India.[24] In this study, the occurrence of staphylococci was studied among the fomites collected from hospitals. All the discarded fomites serves as a potent reservoir of MRSA which may transmit the infection in community and thus the development of community acquired MRSA infection is increasing rapidly. Earlier reports have also suggested that the MRSA strains can survive and be isolated from fomites.[8] Moreover our results seem to agree with the various contemporary investigations.[25-27] Staphylococcal infections are often spread directly by skin contact with a colonized or infected person or animal, but may be spread indirectly through fomites. Even fomites are very vital reservoir of the various infections other than *Staphylococcus* and it depends on the types of fomites and the route from where they are derived. However, environmental sources and fomites cannot be ignored.

*Staphylococcus aureus* is known to have developed resistance to conventional antibiotics[28] and this has been the pattern in our study. The development of antibiotic resistance by antimicrobial agents is troublesome and has been described as a serious public health concern particularly in developing countries.

In the developing country like India, the outbreak of MRSA is very common in various geographical part of the nation.[29] However detection and identification of the MRSA may control the severe epidemic but it needs prompt detection of pathogens. Currently various molecular techniques are used but they are not economical and are unfeasible at small scale.[30,31] Present research proposes the very rapid and cheap method for the detection of pathogen by on-line tools. It is new concept in the present study, which would make identification of the pathogens rapid. The online tool is most fascinating techniques need to implement in the medical microbiology to validate the identification of pathogens. This investigation provides the example to use of such online tool.

Inadequate establishment and availability of new drugs for the MRSA treatment offers the use of antibiotics combination. Even, in developing countries sometimes new therapy and treatments are not available against MRSA, hence combination of various antibiotics are only the option for such country. Tetracycline/sulbactam/ cefoperazone has been far better combination than ampicillin /salbactam/cefoperazone, suggesting the possibility of using combination of new antibiotics than that of older one. Even MIC results of individual antibiotic and mixture of antibiotics also appear similar and are supported by data from other groups.[20,21]

Similarly, various antibiotics combination including cephalosporins and beta-lactamase inhibitors, with cefoperazone/sulbactam has been reported and available across much of Asia, and with ceftriaxone/ sulbactam and cefepime/tazobactam in India.[32] These may be useful in the battle against bacteria with extended-spectrum beta-lactamases (ESBLs).[33] However, apart from the combination of antibiotics for the treatment of MRSA, new treatment options are slowly emerging, including quinolones, ketolides, linezolid, tigecycline and potentially, ceftobiprole and ceftaroline. Outcomes and suggestions of the research also resemble to the revised guidelines for MRSA control. Hence, the present finding has delivered baseline information and documentation which are effective to the epidemiological purpose to control and prevention of drug resistance species.

## 5. Conclusion

Although, number of isolates are less, this study provides evidence that inanimate derived from the hospitals are the potent source of MRSA infection and the significant presence of the infection persist in the society, derived directly and indirectly from the fomites. Proper disposal of fomite and hospital waste is the only way to reduce the infections. This is the strategies to minimize the transmission of infection from fomites. Relatively higher concentration of antibiotics resistance was indicated by the MRSA strains is worrisome indication to the hospital and society.

Treatment using new antibiotics against the MRSA is best way to control them. But it has its own drawback like drug cost and its supply as well as resistance itself. Therefore, it is not successfully being implemented in practice. Even though the use of new antimicrobial agent offers the different resistance mechanisms to the organisms and new resistance will emerged latter. So only promising option for developing country are the use of affordable antibiotics in suitable combination.

## 6. References


1. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, et al. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis. 2007;13:1834-39.doi:10.3201/eid1312.070384 PMid:18258032 PMCid:2876750

2. Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) Strain ST398 is Present in Midwestern U.S. Swine and Swine workers. Plos one. 2009;4:1-6. doi:10.1371/journal.pone.0004258 PMid: 19145257 PMCid: PMC2626282

3. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, Heederik D et al. Methicillin Resistant *Staphylococcus aureus* ST398 in Veal Calf Farming: Human MRSA Carriage Related with Animal Antimicrobial Usage and Farm Hygiene. PLoS ONE 2010;5:1-6.doi:10.1371/journal.pone.0010990 PMCid:PMC2882326

4. Nguyen DM, Mascola L, Bancroft E. Recurring methicillin-resistant *Staphylococcus aureus* infections in a football team. Emerg Infect Dis 2005; 11:526-32. PMid:15829189

5. Oller AR, Mitchell A. *Staphylococcus aureus* recovery from cotton towels. J Infect Developing Countries. 2009; 3:224-28. PMid:19759479

6. Leonas KK, Jinkins RS. The relationship of selected fabric characteristics and the barrier effectiveness of surgical gown fabrics. Amer J Infect Control. 1997; 25:16-23. PMid:9057939

7. Sattar SA, Springthorpe S, Mani S, Gallant M, Nair RC, Scott E, et al. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model. J Appl Microbiol. 2001; 90:962-70. doi:10.1046/j.1365-2672.2001.01347.x PMid:11412326

8. Neely AN, Maley MP. Survival of *Enterococci* and *Staphylococci* on hospital fabrics and plastic. J Clin Microbiol. 2000; 38:724-26. PMid:10655374 PMCid:PMC86187

9. Lankford MG, Collins S, Youngberg L, Rooney DM, Warren JR, Noskin GA. Assessment of materials commonly utilized in health care: implications for bacterial survival and transmission. Amer J Infect Control. 2006; 34:258-63. doi:10.1016/j.ajic.2005.10.008 PMid:16765202

10. Scott EA, Duty S, McCue K. A critical evaluation of methicillin-resistant S. *aureus* and other bacteria of medical interest on commonly-touched household surfaces in relation to household demographics. Am J Infect Control. 2009; 37: 447-53. doi:10.1016/j.ajic.2008.12.001 PMid:19361887

11. Kotsanas D, Scott C, Gillespie EE, Korman TM, Stuart RL. What's hanging around your neck? Pathogenic bacteria on identity badges and lanyards. Med J Aust. 2008; 188:5-8. PMid:18205553

12. Treakle AM, Thom KA, Furuno JP, Strauss SM, Harris AD, Perencevich EN. Bacterial contamination of health care workers' white coats. Am J Infect Control. 2009; 37:101-05. doi:10.1016/j.ajic.2008.03.009. PMid:18834751 PMCID: PMC2892863

13. Huang R, Mehta S, Weed D, Price CS. Methicillin-resistant *Staphylococcus aureus* survival on hospital fomites. Infect Control Hosp Epidemiol. 2006; 27:1267-69. doi:10.1086/507965 PMid:17080391

14. Nourkhoda Sadeghifard, Reza Ranjbar, Javad Zaeimi, Mohammad Yousef Alikhani, Sobhan Ghafouryan, Mohammad Raftari, et al. Antimicrobial susceptibility, plasmid profiles, and RAPD-PCR typing of *Acinetobacter bacteria*, Asian Biomedicine. 2010; 4:901-11. http://www.asianbiomed.org/AutoJournal.php

15. Massey RC, Horsburgh MJ, Lina G, Hook M, Recker M. The evolution and maintenance of virulence in *Staphylococcus aureus*: a role for host-to-host transmission? Nat. Rev. Microbiol. 2006; 4:953-58. doi:10.1038/nrmicro1551 PMid:17109032

16. Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, et al. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacteriol. 2005; 187:2426-38. doi:10.1128/JB.187.7.2426-2438.2005. PMid:15774886 PMCid:PMC1065214

17. Mangili A, Bica I, Snydman DR, Hamer DH. Daptomycin resistant, methicillin-resistant *Staphylococcus aureus* bacteremia. Clin. Infect. Dis. 2005; 40:1058-60.

18. Skiest DJ. Treatment failure resulting from resistance of *Staphylococcus aureus* to daptomycin. J. Clin. Microbiol. 2006; 44:655-56. doi:10.1128/JCM.44.2.655-656.2006. PMid:16455939 PMCid:PMC1392696

19. Tsiodras SH, Gold S, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, et al. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet. 2001; 358:207-08.

20. Nakazawa H, Kikuchi Y, Honda T, Isago T, Nozaki M. Enhancement of antimicrobial effects of various antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) by combination with fosfomycin. J Infect Chemother. 2003; 9:304-09. doi:10.1007/s10156-003-0266-2 PMid:14691650

21. Gad Gamal Fadl M, El-Ghafar Abd El-Ghafar F Abd, El-Domany Ramadan AA, Hashem Zeinab Shawky. Epidemiology and antimicrobial resistance of *staphylococci* isolated from different infectious diseases. Braz. J. Microbiol. 2010; 41:333-44.

22. Bronzwaer S, Lonnroth A, Haigh R. The European Community strategy against antimicrobial resistance. Euro Surveill. 2004; 9:30-34. PMid:14762318

23. Lautenbach E, Synnestvedt M, Weiner MG, Bilker WB, Vo L, Schein J, et al. Epidemiology and impact

of imipenem resistance in *Acinetobacter baumannii*. Infect Control Hosp Epidemiol. 2009; 30:1186-92. PMid:19860563

24. Patil R, Baveja S, Nataraj G, Khopkar U. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in community-acquired primary pyoderma. Indian J Dermatol Venereol Leprol. 2006; 72:126-28. doi:10.4103/0378-6323.25637 PMid:16707818

25. Sakaguchi M, Shime N, Fujita N, Fujiki S, Hashimoto S. Current problems in the diagnosis and treatment of hospital-acquired methicillin-resistant *Staphylococcus aureus* pneumonia. J anesth. 2008; 22:125-30. doi:10.1007/s00540-007-0600-4 PMid:18500608

26. Batra R, Eziefula AC, Wyncoll D, Edgeworth J. Throat and rectal swabs may have an important role in MRSA screening of critically ill patients. Intensive Care Medicine. 2008; 34:1703-06. doi:10.1007/s00134-008-1153-1 PMid:18500421

27. Williams C, Davis DL. Methicillin-resistant Staphylococcus aureus fomite survival. Clin Lab Sci. 2009; 22:34-38. PMid:19354027

28. World Health Organization: Overcoming antimicrobial resistance [online] 2000 (cited 2010 Dec 18). Available from: http://www.who.int/infectious-disease-report/2000/

29. Pai V, Rao VI, Rao SP. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates at a tertiary care hospital in Mangalore, South India. J Lab Physicians. 2010; 2:82-84.

30. Tan TY, Corden S, Barnes R, Cookson B. Rapid identification of methicillin-resistant *Staphylococcus aureus* from positive blood cultures by real-time fluorescence PCR. J. Clin. Microbiol. 2001; 39:4529-31. doi:10.1128/JCM.39.12.4529-4531.2001 PMid:11724876 PMCid:PMC88580

31. Fang H, Hedin G. Rapid Screening and Identification of Methicillin-Resistant *Staphylococcus aureus* from Clinical Samples by Selective-Broth and Real-Time PCR Assay. J Clin Microbiol. 2003; 4:2894-99. doi:10.1128/JCM.41.7.2894-2899.2003 PMid:12843018 PMCid:PMC165274

32. Livermore DM, Hope R, Mushtaq S, Warner, M. Orthodox and unorthodox clavulanate combinations against extended-spectrum ß-lactamase producers. Clin Microbiol Infect. 2008; 14:189-93. PMid:18154546

33. Livermore DM. Has the era of untreatable infections arrived? J Antimicrob Chemother. 2009; 64:29-36. doi:10.1093/jac/dkp255 PMid:19675016