

In Vitro Susceptibility of Chloramphenicol Against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Objective: To determine the *in vitro* susceptibility of chloramphenicol against methicillin-resistant *Staphylococcus aureus*.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from January to June 2012.

Methodology: One hundred and seventy four isolates of methicillin-resistant *Staphylococcus aureus* were included in this study using cefoxitin (30 µg) disc for detection. Minimum inhibitory concentration (MIC) of chloramphenicol against MRSA was determined by using E-strip (AB BIO DISK). The susceptibility was determined by swabbing the Mueller-Hinton agar (MHA) plates with the resultant saline suspension of MRSA and applying E-strip of chloramphenicol from AB Biodisk Sweden and determining the MIC of chloramphenicol (in µg/ml). Clinical and Laboratory Standards Institute (CLSI) recommendations of ≤ 8 µg/ml being sensitive, 16 µg/ml as intermediate and ≥ 32 µg/ml as resistant were followed in interpreting the results.

Results: Out of the 174 MRSA isolates, 132 (75.86%) isolates were susceptible to chloramphenicol with MICs of ≤ 8 µg/ml, 38 (21.84%) were resistant ≥ 32 µg/ml while 4 (2.30%) were in intermediate range with MIC of 16 µg/ml.

Conclusion: Chloramphenicol has shown good *in vitro* activity against MRSA and is likely to have a key role in the treatment of MRSA infections providing us a good alternative to newer expensive antimicrobials in resource limited countries.

Key Words: Methicillin-resistant *Staphylococcus aureus*. Chloramphenicol. *In vitro* susceptibility.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen and one of the leading causes of nosocomial and community acquired infections. With the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), the choices of antimicrobials to treat infections caused by such isolates are limited.¹ MRSA associated infections range from superficial skin and soft tissue lesions to more serious systemic and fatal infections such as necrotizing pneumonia, urinary tract infections, osteomyelitis, endocarditis and septicemia.^{2,3}

Chloramphenicol was introduced into clinical practice in 1949 and alongside tetracycline was considered to be a prototypical broad spectrum antibiotic. Chloramphenicol is effective against most gram positive (including most strains of MRSA) and gram negative bacteria including anaerobes.⁴ The resistance and potential side effects of the drug have largely forced the clinicians to refrain

from using the drug during 70's to 90's. The potential threat of superbugs like MRSA, vancomycin resistance *Enterococci* (VRE) and extended spectrum beta lactamase (ESBL) producing organisms and limitation in choices of antibiotics to treat such infections has forced the researchers to find antimicrobials with activity against such multidrug resistant bacteria. Chloramphenicol, an old antibiotic, suddenly came out of wilderness with renewed interest and is again being considered as an alternative to treat such infections.⁴ Chloramphenicol has wide antimicrobial spectrum and excellent tissue penetration and is used empirically in the hospital setting for the treatment of patients with unknown sources of fever.⁵

Recent studies carried out to determine susceptibility of MRSA against chloramphenicol has revealed very encouraging results. Studies carried out in Pakistan, China, Iran and Nepal has revealed that more than 90% of MRSA isolates in respective set ups isolated from different clinical material were susceptible to chloramphenicol.^{1,2,6,7} On the other hand, there are few studies, one from Pakistan and another from Nigeria (sensitivity of 52.5%) which do not report as effective results as reported from some other countries.^{8,3}

Since MRSA infections are always a challenging problem for treating clinicians, there is strong need in resource limited countries to review the utility of conventional antibiotics for the management of MRSA

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as new agents are expensive and not easily available. There is very limited literature published in our country evaluating the usefulness of chloramphenicol against MRSA.

The objective of the study was to determine *in vitro* susceptibility of chloramphenicol against methicillin-resistant *Staphylococcus aureus*, using interpretation of zones of inhibition by E-strip (AB Biodisk, Sweden) method on Mueller-Hinton agar.

METHODOLOGY

This study was carried out at the Department of Microbiology Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January to June 2012. Permission from institutional ethical and research committee was taken. Sampling technique was non-probability consecutive sampling. All MRSA isolates recovered from clinical specimens were included. No discrimination was made on age and gender of the patient. Clinical isolates other than MRSA, duplicate samples, repeat specimens of same patient and contaminated specimens were excluded. One hundred and seventy four isolates of methicillin-resistant *Staphylococcus aureus* were included in this study. Sample size was calculated by using WHO sample size calculator with anticipated population proportion 52%, absolute precision 10% with 95% confidence level.³ More than 100 isolates were required for the study. Details regarding type and place of submission of specimens were noted and recorded. These micro-organisms were isolated from patients admitted in different wards of Combined Military Hospital, Armed Forces Bone Marrow Transplant Centre and Armed Forces Institute of Urology. The characterization of MRSA was done by using cefoxitin (30 µg) disc and following the interpretation criteria of Clinical and Laboratory Standards Institute (CLSI).⁹

The MIC's of chloramphenicol against these isolates were done after swabbing the Mueller-Hinton agar (MHA) plates with the 0.5 McFarland standard suspension of MRSA and then applying E-strip of chloramphenicol from AB Biodisk, Sweden, and MIC of chloramphenicol (in µg/ml) was noted. E-strips were made available by the institute. However, there was no conflict of interest of authors with the E-strip provider company or any financial or other gains were obtained from the company. Methicillin-resistant *Staphylococcus aureus* ATCC control strain no. 33591 was used as control. CLSI recommendation of MIC ≤ 8 µg/ml was taken as susceptible, 16 µg/ml as intermediate and ≥ 32 µg/ml as resistant.⁹

The data was entered in Statistical Package for Social Sciences (SPSS), version 17 software. Descriptive statistics were calculated for both qualitative and quantitative variables. Qualitative variables like chloram-

phenicol susceptibility, frequency and percentages were calculated. Qualitative variables are presented as tables.

RESULTS

A majority of MRSA isolates (85.63%) were recovered from pus and pus swab while MRSA isolates from other clinical specimens are depicted in Table I.

MICs of chloramphenicol against MRSA isolates revealed that 132 (75.86%) isolates were susceptible to chloramphenicol, 38 (21.84%) as resistant while 4 (2.30%) were having intermediate susceptibility.

Among susceptible lot, a total of 101 (76.52%) isolates had MIC's between 2 – 4 µg/ml while 22 (16.66%) were in the range of 6 – 8 µg/ml and 9 (6.82%) had MIC of 1.5 µg/ml (Table II).

Table I: Isolation of MRSA from different specimens (n = 174).

Specimen type	Number of specimens	Percentage
Pus and pus swab	149	85.63%
Blood	10	5.75%
Tissue	3	1.73%
Catheter tip	3	1.73%
Others	9	5.17%

Table II: MIC of susceptible isolates (n = 132).

MICs	No of isolates	Percentage
1.5	9	6.82%
2	27	20.45%
3	47	35.61%
4	27	20.45%
6	9	6.82%
8	13	9.85%

DISCUSSION

One of the overriding microbial threats of the 21st century has emerged in the form of increased antimicrobial resistance worldwide.^{1,3,4} Multidrug resistant isolates has been reported with varying degree of susceptibility around the globe.^{1,10} Infections due to MRSA are widespread throughout the world and are the major cause of health care associated morbidity and mortality since its emergence in 1961.¹⁰⁻¹³ During the last two decades, epidemiology of this pathogen has changed globally and infections caused by it have also emerged in the community, this emergence and dissemination of MRSA had led to major therapeutic and infection control challenges.^{1,14}

The increased incidence of MRSA globally in general and in Pakistan in particular has led to restricted therapeutic options for clinical isolates. Treatment options include macrolides, aminoglycosides, cotrimoxazole, clindamycin, tetracycline, fusidic acid, quinolones, chloramphenicol, linezolid, and vancomycin. Prediction of susceptibility to these antibiotics requires

knowledge of antibiotic susceptibility pattern of MRSA from a particular region as it varies a lot from region to region and also in continents.¹⁵⁻²¹ In this study, 21.84% of the tested isolates were resistant to chloramphenicol which is comparable to India where 28.6% of the isolates were resistant.¹¹ Other studies conducted in China, Iran and Nepal have revealed surprisingly low resistance rates of 0.8%, 0% and 5.15% resistance respectively.^{2,6,7}

Previously only two studies have been conducted in Pakistan showing the susceptibilities of chloramphenicol against MRSA isolates. The study conducted at the Aga Khan University Hospital revealed that only 10% of MRSA isolates recovered from skin and soft tissue infections were resistant to chloramphenicol which is very encouraging finding.¹ On the contrary, a study conducted at Dow Medical College, Karachi, revealed quite contrasting results with 93% of MRSA isolates being resistant to chloramphenicol.⁸ The present results are definitely in concordance with the results depicted by the Aga Khan University, but the gross diversity in the susceptibility to the antimicrobial in the same region could very well be a possibility reflecting the local antibiotic prescribing practices.

The studies carried out in European countries have revealed that in Greece 100% of the MRSA isolates from community acquired infections were susceptible to chloramphenicol¹⁴ while in UK almost 92.3% of such isolates recovered from patients of otitis externa were sensitive to this antimicrobial.¹⁷ The results from studies carried out in Japan and Korea have also revealed similar pattern as 91.6% and 100% of MRSA isolates were susceptible to this compound respectively.^{19,21} The published literature from USA has revealed that although *in vitro* efficacy of chloramphenicol against MRSA is still very encouraging but there is declining trend noted from different regions of the country.^{22,23} Only one study was found from African country of Uganda where 88.2% of the isolates were susceptible.¹⁶

The most significant finding of our results was the fact that about 75% of the isolates susceptible to chloramphenicol had MIC's of 2-4 µg/ml which is well below the recommended MIC's of 8 µg/ml by CLSI. This result could be attributed to the fact that chloramphenicol is not being routinely used in clinical practice to treat majority of infections caused by gram positive and gram negative bacteria.

Bone marrow toxicity is the major complication of chloramphenicol. This side effect may occur as either due to dose related bone marrow suppression or idiosyncratic aplastic anaemia. This complication is predisposed by high dose (4 g/day), prolonged therapy, and markedly elevated levels in serum (20 mg/ml) and is reversible. The second form of rare complication may manifests as aplastic anaemia. Gray baby syndrome

may occur in premature infants and neonates. This toxicity results from the immature hepatic function of neonates, which impairs hepatic inactivation of the agent.²⁴

Keeping in view the low cost and oral preparation of chloramphenicol coupled with very high rate of *in vitro* susceptibility makes this antimicrobial an ideal choice for wide variety of infections caused by MRSA. Further studies focusing more on the clinical outcome of patients of MRSA treated with chloramphenicol would definitely give icing on cake for *in vitro* results achieved for this compound. It is also imperative that since this compound has shown very promising results against MRSA isolates, the availability of this antibiotic must be ensured in the market for the benefit of patients.

The main limitation of the study was that it showed results from restricted geographic area/region and was purely laboratory based and there was no clinical correlation to see the therapeutic outcome of the drug. It would be more beneficial if multicentre studies are carried out to find out the susceptibility of MRSA isolates against chloramphenicol.

CONCLUSION

Chloramphenicol has shown very good *in vitro* susceptibility against MRSA and is likely to have a key role in the treatment of infections caused by MRSA. This antimicrobial can serve as an alternative to new expensive antimicrobials in resource poor countries. There is need to further evaluate this antimicrobial for determining the *in vitro* as well as *in vivo* efficacy before broad based usage of this compound can be undertaken.

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