# DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES PRODUCING MRSA FROM HOSPITAL WASTE WATER IN LAHORE

**Sumbal Sarwar[1], Komal Saleem[1], Warda Fatima[1*], Anjum Nasim Sabri[1]**

[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan

Corresponding Author: Warda Fatima E. mail: warda.mmg@pu.edu.pk

***ABSTRACT:*** *Staphylococcus aureus is one of the major sources of nosocomial infections in humans. Its increasing resistance to different antibiotics and ubiquitous presence is important cause of spreading infections. Methicillin is modified β lactam antibiotic used to cure infections by this organism but due to presence of mecA gene in plasmid, S. aureus has developed resistance to methicillin. Treatment of methicillin resistant S. aureus (MRSA) is difficult and can be done by vancomycins. Extended spectrum β lactamases also pose difficulty in treatment by different drugs as they are also resistant to a number of antibiotics. In this study, the waste water from a public sector hospital was taken and was checked for the presence of S. aureus. A number of antibiotics including third generation cephalosporins and methicillin were used to find out bacterial sensitivity to these drugs and one of the strains was found to be resistant to methicillin as well as cephalosporins showing that ESBLs producing MRSA can be found in waste waters. These can cause spread of resistant bacteria and complex diseases. So it is necessary that hospital wastes should be checked for the presence of pathogenic bacteria and proper eradication of infectious material should be done before disposal.*

**Key Words:** Antibiotic susceptibility, Double disc Synergism, Extended spectrum β lactamases, Methicillin resistance

## INTRODUCTION

*Staphylococcus aureus* is a member of human normal flora, specifically present on skin but it can be pathogenic if gets entered inside the human body and may cause many diseases from simpler (pneumonia) to complex (septicemia or endocarditis) [1, 2]. It is also important in nosocomial as well as community acquired infections [3, 4].

Mostly *S. aureus* associated infections are treatable with β lactam antibiotics that target biosynthesis of the essential component of bacterial cell wall that is peptidoglycan. It increases cell permeability and creates imbalance of flow of materials in and out of cell leading to cell death [5]. By the passage of time and extensive abuse of antibiotics, this bacterium has started getting resistance against these drugs. Starting from first report on β lactam (penicillin) resistant *S. aureus* there is a continuous search for options to reduce and stop the spread of resistance [6]. For this purpose Methicillin -- a modified β lactam that has special site for blockage of β lactamase was developed in 1960. But soon after its development methicillin resistant *Staphylococcus aureus* (MRSA) were also observed [6, 7].

The most common reason behind this methicillin resistance is the possession of *mecA* gene in the resistant strains. *MecA* gene produces a modified penicillin binding protein (PBP2A). β lactamase cannot bind to these altered sites and thus become less effective in disrupting the cell walls [6]. Still the number of bacteria with such high resistance is low and most of *S. aureus* infections are treatable with many β lactams.

Cephalosporin also come under the category of β lactams, there are five generations of this antibiotic and many of its members are active against bacteria, although bacteria harboring the enzyme cephalosporinase confer resistance to this class of antibiotics too. Extended spectrum beta lactamases are one of the most important causes of resistance to penicillin and cephalosporins in many organisms. ESBL producing bacteria are also frequently resistant to aminoglycosides, trimethoprim sulfamethoxazole and quinolones, reducing the available treatment options [8]. However, study during the last two decades found that bacterial resistance mediated by plasmids which carry resistance gene to a large number of antibiotics, which are rapidly transferred has worsened the scenario [9, 10].

Infectious materials from hospital wastes, if not treated properly and disposed, and mixes with municipal waste can contaminate the whole waste with infectious agents [2]. Hospital waste water can be an obvious source of many pathogenic bacteria as they carry the resistant genes [11]. If the waste from hospitals gets mixed with drinking water or is discharged without treatment it can cause many infections and can also become a source for emergence of antibiotic resistance among bacterial species prevailing in an environment.

For the purpose of identifying MRSA in waste water of hospital and to check its sensitivity against drugs this study was performed.

## MATERIALS AND METHODS

### Sample collection

Water sample from the waste water disposal tank of a public sector hospital from Lahore was collected in aseptic conditions in sterile container and was transferred to laboratory. Temperature and pH at site was noted and within 3 hours of collection sample was treated further.

### Isolation and identification:

Sample was inoculated with serial dilutions on Nutrient agar and blood agar plates. Isolated colonies were purified on Mannitol salt agar plates to obtain maximum strains. Plates were incubated at $35^0$C for 24 hours. Different colonies that appeared after 24 hours were differentiated on basis of color and yellow colonies with yellow zones around them were selected for further biochemical testing. Gram staining, catalase, DNAse enzyme activity and gelatin hydrolysis tests were carried out to screen *S. aurues*.

### Effect of physical factors

Effect of physical factors like temperature and pH was noted on the growth of biochemically identified *Staph. aureus* strains. First of all 1% Macfarland standard was prepared and then bacterial strains were grown in L. broth for 24 hours at different temperatures ($15^0$C – $55^0$C) to check the effect of

temperature on growth. After that optical densities of cultures were taken at 600 nm. To check pH effect on bacterial growth, strains were inoculated in L. broth medium with different concentrations of acid and alkali forming the pH range of 4-9 and incubated at $35^{O}$C for 24 hours. After that optical densities were taken at 600 nm.

**Antibiotic susceptibility testing**

Bacterial strains were treated with 10µg methicillin for confirmation as MRSA by placing the discs of this antibiotic on bacterial lawn on Muller-Hinton (MH) agar and incubating it on $37^{0}$C for 24 hours.

For checking the production of cephalosporinase enzyme bacterial lawn was prepared on MH agar and 30µg discs of Ceftazidime, Cefuroxime, Cefotaxime and Aztreonam were placed for 24 hours on $37^{0}$C. Inhibition zones were checked by mm scale manually. Three plates for each antibiotic were checked and their mean was taken.

For double disc synergism, 30µg concentration amox + calvulanic acid (augmentin) was used along with 30µg concentration each of Cefotaxime, Ceftazidime, Aztreonam, Cefuroxime and Ceftriaxone.

## RESULTS

**Isolation and characterization:**

In October 2013, 450 ml water sample from the waste water disposal tank from a public sector hospital in Lahore was taken. At site temperature and pH was noted that was $27^{0}$C and 6.5, respectively. Dilutions of sample were prepared and plated on N. agar and blood agar plates. Purification of isolated colonies was done on mannitol salt agar medium to find out different Staphylococcus species present in the sample. The plates were placed on $35^{O}$C for 24 hours and then checked for production of different colonies. As *S. aureus* gives yellow colonies while *S. epidermidus* forms red color colonies on this medium. So it was easy to differentiate only *S. aureus* and treat them further. The yellow colonies, on microscopic examination revealed the presence of staphylococci that were positive for Gram stain; catalase production; hydrolysis of DNA and hydrolysis of gelatin so were characterized and confirmed as *S. aureus*. Following this procedure two *S. aureus* strains were isolated and used in further experimentation.

**Effect of Physical factors**

To check the optimal growth conditions for bacterial isolates, effect of pH and Temperature was noted and it was noted that best growth of *S. aureus* isolates was on pH 7 and temperature $35^{0}$C (Table 1)

**Table 1: Effect of changing physical conditions on S. aureus**

| No | pH | OD+ | No. of cells* | Temp. | OD+ | No of Cells* |
|---|---|---|---|---|---|---|
| **1** | 4 | 0.044 | $4.4X10^{7}$ | $15^{0}$C | 0.08 | $8X10^{8}$ |
| **2** | 7 | 1.259 | $1.2X10^{8}$ | $35^{0}$C | 1.32 | $1.32X10^{9}$ |
| **3** | 9 | 0.339 | $3.3X10^{8}$ | $55^{0}$C | 0.86 | $8.6X10^{8}$ |

+=OD is optical density taken at 600 nm

*= OD 1 at 600 nm is equal to $10^{9}$ bacterial cells

**Antibiotic susceptibility testing:**

Isolated strains were tested for methicillin resistance by adding disc of methicillin on MH agar and one of the strains showed resistance to methicillin (Figure 1). This strain was tested further with other β lactam antibiotics including Cefatoxime, Ceftazidime, Aztreonam, Cefuroxime and Ceftriaxone. Results revealed that one of the isolated strains was resistant to all of the antibiotics tested (Figure 2)

These antibiotics were again tested for synergistic effect on MRSA and for this purpose double disks for cefotaxime, ceftazidime, aztreonam, cefuroxime and ceftriaxone were applied in synergism with amoxicillin and clavulanic acid. Sensitivity zone for microbe was increased by addition of these antibiotics combined (Figure 3).

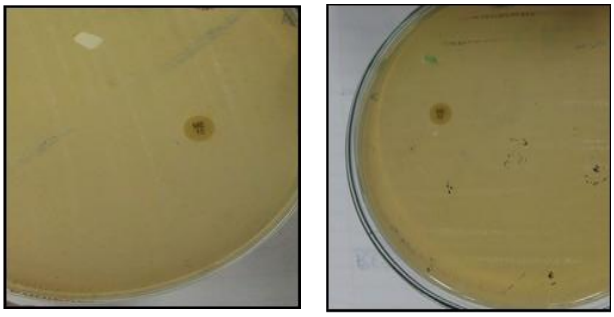

**Figure 1: Antibiotic susceptibility testing: Methicillin antibiotic 10ug conc. Disc. Both plates show *S. aureus* resistance to antibiotic after incubation.**

## DISCUSSION

Multiple drug resistance among bacteria is an emerging global issue. Over the past 10 years resistance of Gram positive bacteria is the major issue in clinical and nonclinical settings [12]. One environmental concern related to hospital waste water which was overlooked from so many years is its discharge without any effective treatment.

Hospital waste water is most hazardous to public health as it contains most pathogenic form of bacteria. Studies confirm the survival of bacteria in minimal nutrient environment including hospital waste water. Sources of liquid hospital waste mainly include the indoor and outdoor departments, pathology laboratory including microbiological sections which contain viable cultures, stocks, diagnostic samples and operation theatres.

Hospital waste disposal is very important and crucial matter as if the municipal waste gets contaminated with hospital waste then it would lead to the spread of multiple drug resistant organisms [11]. Our experimentation was done in October 2013 and it was noted that from the waste water disposal tank of public sector hospital from Lahore, ESBL producing MRSA was isolated. Isolation of *S. aureus* – a potential pathogen is a common feature from water samples

**Figure 2: Antibiotic susceptibility testing: Discs of 30ug each of Ceftazidime, Cefuroxime, Cefotaxime plated on MH agar medium.Two discs of Cefuroxime were used in experiment . (Sizes of inhibition zones were 6mm, 14mm, and 13 mm respectively. While for Ceftazidime ≤17mm, Cefuroxime ≤14 and for Cefotaxime ≤22mm was considered resistant)**

**Figure 3: Double disc synergism: Discs of 30ug each of Ceftazidime, Cefuroxime, Cefotaxime and Aztreonam were plated on MH agar medium incombination with Amox + Clavulanic acid AMC/AUG**

as this bacterium can survive in water as well as it is common inhabitant of waste waters. But if the resistant bacteria is found in drinking waters then it is possible that spread of such bacterium in waters can cause the severe diseases that may lead to serious public health problems.

Our findings were in consistence with studies of Ahaduzzaman et al. [13], Alamgir and Ahsan, [14] and Bukhari et al. [15] who all have reported the resistance of bacteria, that were isolated from waste materials, to many antibiotics including methicillin and β lactamas.

Samina et al. [16] have found that in Pakistan both private and public sector hospitals lack the proper disposal of waste materials that can not only cause environmental pollution, unpleasant smell, growth and multiplication of insects and worms but may also lead to the transmission of diseases and spread of resistant pathogens.

## CONCLUSION

It can be concluded from this study that waste disposal tanks of public sector as well as private sector hospitals should be properly decontaminated before disposing hospital waters in municipal waters. For, if there are some resistant microbes present they can cause spread of resistance genes among general microbial population and severe diseases in human population can emerge.

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