

## Prevalence of Antibiotic Resistant *Staphylococcus aureus* Among Patients who Comes to Seek Treatment in a Hospital of Bangladesh

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### Abstract

Methicillin-Resistant *Staphylococcus Aureus* (MRSA) infections now become threat and have spread worldwide. This can be very serious and are among the most frequently occurring of all antibiotic-resistant threats. The antibiotic resistance problem has been attributed to the misuse or overuse of these medications, as well as a lack of new drug development by the pharmaceutical company. In this study total 230 outdoor & indoor patients in Gonoshasthya Nagor Hospital, Dhaka, Bangladesh during May 2016 to May 2017 were enrolled to detect MRSA. For this study 8 types of biological specimen (Urine, Pus, Blood, Sputum, Swab (Ear/Throat/High vaginal) and Stool) were collected and screening for antibiotic resistance against seven (ampicillin, erythromycin, tetracycline, ciprofloxacin, gentamicin, cephalixin and penicillin) commonly used locally available antibiotics. Among 230 total 70 samples (30.4%) were found at least resistant to one drugs while drug resistance pattern was Amoxicillin, Erythromycin, Ciprofloxacin, Ceftriaxane, Cloxacillin, Cephalixin and Gntamicin.

**Key Words:** Methicillin-Resistant *Staphylococcus Aureus* (MRSA); Antibiotic resistance and health care-associated infections

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### Introduction

*Staphylococcus aureus* is a Gram-positive, nonmotile, coagulase-positive coccoid bacterium of the Firmicutes phylum. *S. aureus* is found in the human commensal microbiota of the nasal mucosa in 20–40% of the general population (Lee *et al.*, 2018). But Methicillin-Resistant *Staphylococcus Aureus* (MRSA) infections now become threat and have spread worldwide. MRSA infections can be very serious and are among the most frequently occurring of all antibiotic-resistant threats (Carlet *et al.*, 2012; Gross, 2013). The antibiotic resistance

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problem has been attributed to the misuse or overuse of these medications, as well as a lack of new drug development by the pharmaceutical company (Lushniak, 2014; Wright, 2014). The Centers for Disease Control and Prevention (CDC) has classified a number of bacteria as presenting urgent, serious, and concerning threats (Rossolini, Arena, Pecile, & Pollini, 2014).

After first identification in 1961, MRSA was found responsible for several hospital outbreaks (health-care-associated MRSA) in many parts of the world (Chambers & DeLeo, 2009). A substantial change in MRSA epidemiology was observed when it was detected in individuals without previous health-care contact (referred to as community-associated MRSA), notably among indigenous populations in Australia in the 1980s (Faoagali, Thong, & Grant, 1992) and otherwise healthy persons, including children, in the United States in the 1990s (Fridkin *et al.*, 2005). Since the mid-2000s, it has also been associated with livestock exposure (livestock-associated MRSA) (Lee *et al.*, 2018).

However, a number of drugs are still activity used against MRSA, including glycopeptides (e.g., vancomycin and teicoplanin), tige-cycline, linezolid, daptomycin, and even some new beta-lactams, such as ceftobiprole and ceftaroline (Rossolini *et al.*, 2014). However, MRSA has shown good adaptability at emerging and spreading in different epidemiological settings over time (in hospitals, the community, and, more recently, in animals). This compounds the epidemiology of MRSA infections and creates a challenge for infection-control systems that focus only on health care-associated infections (HAIs). But fortunately, the incidence of HAI MRSA infections seems to be declining, since aggressive preventive hygiene measures in hospitals (Rossolini *et al.*, 2014).

MRSA can cause a wide range of infections, such as SSTIs, pneumonia, osteoarticular infections, toxic shock syndrome (a rare, potentially life-threatening complication of infection with certain types of bacteria, including *S. aureus*, caused by the release of bacterial toxins and presenting with clinical features that can include fever, rash and hypotension) and bacteraemia, which may be complicated by endocarditis or severe sepsis (Lee *et al.*, 2018; Lowy, 1998).

Individuals who have had extended hospitalization, ICU admission, residency in a nursing home, antibiotic exposure, haemodialysis, surgery, chronic wounds or indwelling invasive devices have an increased risk of infection with healthcare-associated MRSA (Epstein *et al.*, 2015). In addition, asymptomatic colonization with MRSA is a risk factor for subsequent infection, as individuals with MRSA colonization on admission had a relative risk of infection of 13 (95% CI 2.7–64.0) compared with those with MSSA colonization or 9.5 (95% CI 3.6–25.0) compared with those without *S. aureus* colonization (Davis, Stewart, Crouch, Florez, & Hospenthal, 2004). This study was design to identification MRSA among patient who comes to seek treatment in a hospital.

## Methodology

### Study Design

Total 230 outdoor & indoor patients in Gonoshasthya Nagor Hospital, Dhaka, Bangladesh during May 2016 to May 2017 were selected for this descriptive cross-sectional study. Patients consent and information were collected but Name of the participator or any personal identifying information were not recorded in the questionnaire instead a unique identification number.

### Study specimen collection and processing

For this study 8 type of biological specimen were collected and processed as following

**Urine:** Urine samples were collected in a sterile, dry, leak-proof container and mixed with boric acid powder (20 g/l) was added to preserve the specimens and mixed well. The containers were then labeled and microbiological examinations were performed within 48 hours.

**Pus:** Specimens were collected using a sterile cotton-wool swab stick and inserted it in a labeled container of Amies Transport Medium. Microbiological examinations were performed within six hours.

**Blood:** 5 ml patient's blood was collected with sterilized one time syringe before the use of any antibiotic therapy and introduced aseptically into a blood culture bottle.

**Sputum:** Sputum was collected in a clean, dry, wide-necked, leak proof labeled container and examine within six hours.

**Swab (Ear/Throat/High Vaginal):** Specimens were collected using a sterile cotton- wool swab stick and inserted it in a labeled container of Amies Transport Medium. Microbiological examinations were performed within six hours.

**Stool:** Samples were collected in PBS containing container and microbiological examinations were performed within 48 hours of collection.

Total 100 µl of samples was spread over nutrient agar plate surface by a spreading glass rod and then incubated at 37°C for 24 hours. After 24 hours, plates containing ≥30 colonies were selected for counting with dark field Quebec colony counter.

#### **Identification of *Staphylococcus aureus***

*Staphylococcus aureus* ATCC-25923 was used as control and *S. aureus* isolates were identified on the basis of morphology and biochemical test recommended by Buchnon and Gibbons (Bergery, Buchanan, & Gibbons, 1974).

**Morphological studies:** With an aim to identify the selected strains, the following morphological characteristics were carefully studied. To identify *S. aureus* isolates from Nutrient agar and Blood agar media; shape, edge, elevation, opacity and color of the bacterial colony were studied after 24 hour incubation at 37°C.

**Microscopic study:** Gram staining, Acid-fast staining and Spore staining technique were applied for Microscopic examination of bacteria according to Cowan and Steel (Cowan & Steel, 2004).

**Biochemical studies of the selected strains:** After morphological and microscopic examination suspected *S. aureus* colony were selected for following biochemical test coagulase test, catalase test, indole production, methyl red test, Voges-proskauer reaction, urease production, citrate utilization and sugar fermentation according to Bergeys Manual of systematic Bacteriology (Bergery *et al.*, 1974; Cowan & Steel, 2004).

**Antibiotic sensitivity determination:** Antibiotic Resistance of the isolates was determined against seven (ampicillin, erythromycin, tetracycline, ciprofloxacin, gentamicin, cephalixin and penicillin) commonly used locally available antibiotics. To prepare disc, antibiotic solution of different concentrations (30, 50, 75 and 100 µg/ml) were soaked by a small pieces of filter paper, marked as per concentration. Disc-Diffusion Agar method was applied to measure antibiotic sensitivity of all isolates and after overnight incubation, the zone diameters were measured.

## **Result**

Samples were collected from outdoor & indoor patients most of them were women (67%) and age between 18-30 years old (Table 1). Among 230 total 70 samples (30.4%) were positive for *Staphylococcus aureus* infection which were subjected for resistance test (Table 2).

Age Group	Total patients	Male	Female	Positive	Negative
Infant (1 Day-06 Years)	1	00	01	0	01
Child (06-12 Years)	2	01	01	0	02
Young (12-18 Years)	14	05	09	6	08
Youth (18-30 Years)	160	52	108	50	110
Adult (30-50 Years)	39	12	27	11	28
Senior Citizen (50+ Years)	14	06	08	03	11
Total	230	76 (33%)	154 (67%)	70 (30.4%)	160 (69.6%)

**Table 1:** Sample demography.

Name of drug	Sensitive*	Resistant***	Intermediate**
Amoxicillin	03 (04.2%)	66 (94.2%)	01 (01.6%)
Erythromycin	46 (65.7%)	18 (25.8%)	06 (08.5%)
Cephalexin	38 (54.4%)	20 (28.5%)	12 (17.1%)
Ciprofloxacin	09 (12.5%)	59 (84.5%)	02 (03.0%)
Cloxacillin	46 (65.7%)	20 (28.5%)	04 (05.7%)
Gentamycin	56 (80.0%)	11 (15.7%)	03 (04.2%)
Ceftriaxone	63 (90.0%)	05 (07.1%)	02 (02.9%)

**Table 2:** Antibiotic resistance result of *S. aureus* positive (+ve) isolates. (Total Number 70).

\*Sensitive = Colony which show clear zone around 30 ug/ml Antibiotic disc.

\*\*Intermediate = Colony which grow around 30 ug/ml Antibiotic disc but show clear zone for 50 ug/ml Antibiotic disc.

\*\*\*Resistant = Colony which grow around 50 ug/ml Antibiotic disc or higher concentration of That.

Surprisingly all 70 isolates were found at least resistant to one drugs while drug resistance pattern was Amoxicillin, Erythromycin, Ciprofloxacin, Ceftriaxane, Cloxacillin, Cephalexin and Gntamicin.

## Discussion

Rapidly emerging resistant bacteria threaten the extraordinary health benefits that have been achieved with antibiotics (Bartlett, Gilbert, & Spellberg, 2013). Antibiotic-resistant infections place a substantial health and economic. Coordinated efforts to implement new policies, renew research efforts, and pursue steps to manage the crisis are greatly needed.

A study conducted in rural community in Bangladesh, which showed 75 drug purchases were made per 1000 residents per week; where 26% were antibiotics and 9% were of tetracycline. This study also showed 48% of the antibiotics for persons aged 15 years or more and purchased in quantities which represented less than a single day's dose (Hossain, Glass, & Khan, 1982). These practices have probably not led to improvements in health and may have promoted the emergence and persistence of drug-resistant micro-organisms.

Beside this study conducted by Hossain, *et al.* (Hossain *et al.*, 1982) several study was point out miss use of antibiotic in low or middle income country (Istúriz & Carbon, 2000; Kunin *et al.*, 1987; Okeke, Lamikanra, & Edelman, 1999; Radyowijati & Haak, 2003) including Bangladesh.

MRSA control interventions have been widely implemented across health-care facilities. These interventions aim to limit the emergence of MRSA by facilitating judicious use of antimicrobial agents (including introducing restrictions on their prescription), control

the reservoir of patients who are carriers, prevent MRSA transmission between patients and prevent the development of infection in carriers. Several measures are usually required to successfully prevent transmission and infection with MRSA (Calfee *et al.*, 2014). Decolonization, an important control intervention for which there is growing evidence, is discussed in the Management section (Lee *et al.*, 2018).

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