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A Comparative Analysis of the Multi-Drug Resistance Patterns in S*taphylococcus aureus* Isolated from Various Sources in Dhaka, Bangladesh

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The agar disc diffusion test was performed to determine the activity of selected antibiotics against *Staphylococcus aureus* strains obtained from various sources in Dhaka, Bangladesh. The 80 *S. aureus* strains used were isolated from 4 categories of sources: milk and food, normal skin microflora of humans, patient samples, and hospital environment swabs; 20 samples were obtained from each category. Ten antibiotics commonly used for various purposes were chosen: penicillin-G, ampicillin, streptomycin, tetracycline, amoxicillin, neomycin, ciprofloxacin, vancomycin, cefaclor, and bacitracin. It was seen that 1.25% (vancomycin) to 82.5% (penicillin) of the *S. aureus* strains are resistant to the antibiotics used against them. Patient and hospital environment samples exhibited a higher resistance rate than food and normal microflora isolates. Multi-drug resistance, defined as resistance to penicillin and 4 other groups of antibiotics, was found in 35% of the isolates. Of these, three of the hospital environment samples were resistant to at least 8 antibiotics, including one isolate which was vancomycin-resistant. Over 40% of the strains are resistant to 5 or more of the antibiotics tested. 80% of the isolates tested were susceptible to ciprofloxacin, vancomycin, cefaclor and bacitracin.

Key words: Analysis, multi-drug resistance, *Staphylococcus aureus.*

Staphylococcus aureus occurs as a commensal on human skin, particularly the scalp, armpits, and nasopharynx; its primary habitat is the moist squamous epithelium of the anterior nares [1]. Nasal carriage occurs in 40-50% of humans[2] .

This organism is also capable of growth in a wide variety of foods, and especially in foodstuffs that require manipulation in processing, such as in fermented foods and milk products like cheeses[3]. It is one of the most common causes of gastroenteritis but in most countries, its presence in low degrees (e.g. 10^3 cfu/ g of food) is tolerated as it is not a threat to public health.

S. aureus also occurs widely on environmental surfaces and is particularly of concern in hospital environments because of its presence in bed sheets, clothing, and other fomites[4,5].

S. aureus is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance[3,6]. Over the past several decades, it has emerged as one of the most important human pathogens and has become a leading cause of nosocomial infections[4,7]. It may cause a variety of clinical infections with high morbidity and mortality rates; these include wound sepsis, septicemia, pneumonia, osteomyelitis, post-surgical toxic shock syndrome, and septic arthritis[8-11].

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The staphylococci are known to be variably resistant to many antibiotics[4-12]. Until relatively recent reports of low-level glycopeptide resistance[13] and the transfer of high-level vancomycin resistance from *Enterococcus* to *S. aureus*[14], the glycopeptide vancomycin was the last available drug to which this organism had remained uniformly susceptible. They are currently resistant to almost all of the older antibiotics; this crisis has been fueled by the extensive use of antibiotics in the community and hospitals[15].

In many developing countries like Bangladesh, over-the-counter sale of antibiotics is widespread and this accelerates resistance among the common pathogens. Unnecessary prescriptions, e.g. even in the case of viral illnesses in which case antibiotics are ineffectual, are also a contributing factor[16]. In addition, antibiotics are routinely used as growth promoters in farm animal feeds, for prophylaxis and in feed additives in aquaculture[17]. In the case of livestock, it has been noted that they often harbor resistant microflora and excrete them in the milk.

A comparative analysis of the antibiotic resistance patterns of *S. aureus* collected from diverse sources in Dhaka, Bangladesh was carried out in order to evaluate the current status of antibiotic resistance in this organism. Relatively little data exists about such resistance in this part of the world. Emergence of unusual resistance and similar information is invaluable to clinicians in the case of pathogenic strains; in addition, any resistance thought to have arisen from other sources (such as antibiotic overuse in animal feeds) is of concern from public health aspects. Awareness towards antibiotic regulation should be raised in that case. Also, resistance patterns in hospital-environment strains will indicate the severity of nosocomial infections that are likely to occur.

MATERIAL AND METHODS

S. aureus samples were isolated from 4 different categories of sources

20 different samples of milk products such as sweets, baby food, raw milk, and other food products including raw vegetables, were used. 1 g of each sample was suspended in 10 ml of sterile water and shaken well; approximately 1 ml of the resulting solution was spread across the surface of a mannitol-salt agar (MSA) plate.

20 samples were obtained from patients of *S. aureus* infections at Dhaka Medical College Hospital. These were cases of cellulitis, burn infections, and surgical or other wound infections. A sterile cotton swab was used to sample pus and/or the area surrounding the infection in each case, and placed into a test tube of sterile nutrient broth for overnight incubation. The swabs were then streaked over MSA plates.

Sterile cotton swabs were used to collect surface samples from hospital environments, e.g. floors, clothing of personnel, bedspreads, utensils etc. 20 such swabs were placed into sterile nutrient broth tubes and incubated overnight for enrichment. The swabs were streaked over the surface of MSA plates, and incubated.

20 random people contributed the samples for normal microflora; sterile cotton swabs were used to obtain bacteria from skin surfaces such as those between the toes, behind the ears, and the nares. The organisms were enriched by overnight incubation in test tubes of sterile nutrient broth, and then streaked over the surface of MSA plates.

After incubation for 24h, single golden-yellow colonies from each of the plates were subcultured and used as stock cultures. Confirmatory tests for *S. aureus* included the gram stain, catalase and coagulase reactions.

The antibiotic susceptibility of each of the test organisms was determined using the standard disc-diffusion (Kirby–Bauer) method[18] as described below. Cultures no more than 48h old were used in plating. The antibiotic discs (Oxoid®, UK) used in this study were: amoxicillin, 10 µg; ampicillin, 25 µg; bacitracin, 10 µg; cefaclor, 30 µg; ciprofloxacin, 5 µg; neomycin, 30 µg; penicillin-G, 10 units; streptomycin, 10 µg; tetracycline, 30 µg; vancomycin, 30 µg.

For plating each sample, standard aseptic technique and the following procedure was used:

A moderately turbid suspension was prepared by inoculating a loopful of bacterial culture into 1ml of sterile water, and shaking well.

A sterile cotton swab was moistened with the resulting suspension, and streaked all over the surface and around the circumference of MSA in

a Petri plate. Each sample was plated twice.

Using sterile forceps, antibiotic discs were placed equally spread apart on the surface of the medium. 5 discs were used on each plate.

The plates were incubated overnight and the results were obtained no more than 24h from inoculation.

RESULTS

The resistance rate towards each antibiotic was calculated as the number of isolates resistant to that antibiotic divided by the total number of isolates; this is expressed as percentage resistance in the following graph which summarizes the results (Fig. 1).

The percentage of isolates exhibiting multiple-drug resistance, defined as resistance to the penicillins and non-susceptibility to at least three other groups of antibiotics, was calculated and is shown in Table 1.

Fig. 2 showing the resistance rates, expressed as a percentage of the total number of samples, for each of the antibiotics.

Table 1. Percentage of isolates from each group showing multiple-drug resistance

Sample Category	Percentage of Multiply-Resistant Isolates
Food	35%
Clinical	30%
Normal Microflora	35%
Hospital Environment	40%

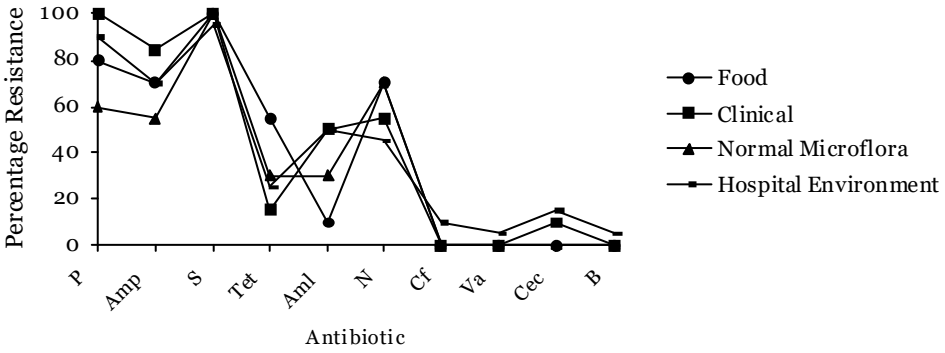

Fig. 1. Percentage resistance of the samples to each of ten antibiotics. [P=Penicillin, Amp=Ampicillin, S=Streptomycin, Tet=Tetracycline, Aml=Amoxicillin, N=Neomycin, Cf=Ciprofloxacin, Va=Vancomycin, Cec=Cefaclor, B=Bacitracin].

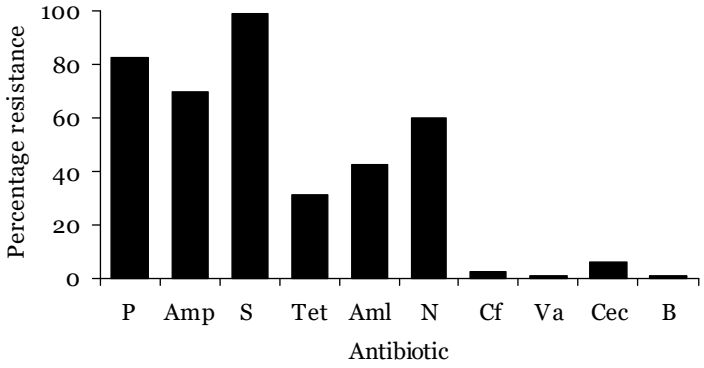

Fig. 2. Percentage of total isolates (n=80) resistant to the antibiotics used.

DISCUSSION

It is evident from this study that penicillin resistance (82.5% of all isolates) is widespread among *S. aureus* regardless of their source; while the relatively newer amoxicillin appears slightly more useful against it than ampicillin or penicillin, it seems likely that that will not remain the case for long, as resistance among clinical and hospital samples are already 50%.

The hospital environment samples show the highest number of multiply-resistant strains and comprise the only group exhibiting resistance to all 10 drugs.

Neomycin is a widely used over-the-counter drug, and the strains are either resistant or intermediate in their response to it, with no susceptibility. This may be an indication of evolving resistance, soon to be followed by complete resistance as in the case of streptomycin. The normal skin microflora show highest resistance toward this drug compared to the others, possibly because of its wide usage in non-prescribed medication in Bangladesh.

Tetracycline shows moderate levels of resistance except in the isolates from milk. The isolates from raw milk and milk products are highly resistant when compared to those from other food products such as vegetables, and this may be a result of the milk being harvested from livestock being fed antibiotics.

Vancomycin resistance was found in one isolate, from a hospital sample. This is rather alarming as it represents 1 vancomycin-resistant strain in 20 hospital samples, and this was previously unreported. Since nosocomial infections by *S. aureus* is very common in Bangladesh, this poses a serious threat.

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