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The Author

List of Abbreviations

ACMSF	Advisory Committee on the Microbiological Safety of Food
AMR	Antimicrobial Resistance
β-lactam	Beta-lactam
BPW	buffered peptone water
CCC	Chittagong City Corporation
CDC	Centers for Disease Control and Prevention
cm	Centimeter
Cep	Cephalothin
Cli	Clindamycin
CLSI	Clinical and Laboratory Standards Institute
CA	community-acquired
ENR	Enrofloxacin
ERY	Erythromycin
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
ESBLs	extended Spectrum β-lactamase
ESBL	Extended-spectrum b-lactamase
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FSA	Food Standards Agency
I	Intermediate sensitive
Kan	Kanamycin
MRSA	Methicillin Resistant *Staphylococcus aureus*
ml	Milliliter
mm	Millimeter
MDR	Multiple Drug Resistance
Oxa	Oxacillin
P	Penicillin
%	Percentage
R	Resistance
S	Sensitive
SEs	staphylococcal enterotoxins
UK	United Kingdom
US	United States
USDA's	United States Department of Agriculture
USFDA	United States Food and Drug Administration
WHO	World Health Organization
XLD agar	Xylose lysine Deoxycholate agar

Abstract

Staphylococcosis is one of the most common and widely distributed food borne diseases, and presence of antimicrobial resistant *Staphylococcus* in food products is a global public health crisis. During the last decade, multi-resistance of *Staphylococcus* spp. has increased, especially in developing countries. Therefore, present study was conducted on randomly selected household and restaurant of Chittagong City Corporation to determine the prevalence of *staphylococcus* spp. and antimicrobial resistance pattern of *Staphylococcus* isolated from gut contents and external washings of cockroach. A total of 50 adult cockroaches, captured aseptically from Three household and four restaurants were identified as *Periplaneta americana*. Culturing external surface wash and gut homogenates by pooling cockroaches, resulted in the isolation of Staphylococcus spp. Examination of *Staphylococcus* spp. on external washings and gut content of cockroach were carried out using standard method and antibiotic susceptibility profiles of the isolates were determined using disc diffusion method during the period July to December, 2014. Out of the 50 sample the highest prevalence was found in gut isolates of households (66.67%) and lowest (40.00%) in both external and gut isolates of household and restaurants and variation in prevalence was not statistically significant ($p>0.05$). Among six antimicrobial tested highest level of resistance found in Penicillin (72.72-85.71%) followed by Erythromycin (45.45-64.28%), Clindamycin (27.27-78.57%), and Oxacillin (21.42 45.45%). The rate of sensitivity to individual antibiotics against *Staphylococcus* isolates was highest in Kanamycin and Cephalothin (100%) followed by Oxacillin (45.45-57.14%), Erythromycin (21.42-27.27%), Penicillin (14.28-27.27%) and Clindamycin (0-7.14%). *Staphylococcus* isolates was multidrug resistant up to four of the six antimicrobials tested. In conclusion, it can be said that the rational use of antibiotics need to be adopt in both human and animals to prevent the emergence of drug resistance *Staphylococcus.*

Key words: Antimicrobial, prevalence, resistance, staphylococcus, cockroach

Chapter-1: Introduction

Staphylococci are ubiquitous organisms which are found most frequently within tissues, on skin surfaces, or in foods contaminated by exposure to infected human beings or animals. There is a close relationship of human and animal pathogenic staphylococci. The public health importance of staphylococcus is a major issue. Cockroach is one of the pests that are closely associated to the food which carries and spread the organism frequently. Cockroaches are some of the most important pests in urban environments (Bennett and Owens, 1986). They are frequently found in large multi-family dwellings where it is difficult to get rid of them completely (Wood, 1980). Their presence can cause many problems as it reduces people's perception of their well-being and the satisfaction they derive from their own personal environment (Wood *et al.,* 1981). In flats, cockroaches are found mainly in kitchens, bathrooms, toilets and cupboards used for storing food. Cockroaches are able to move from one part of a building to another (Robinson *et al.,* 1980; Owens and Bennett, 1982; Rivault, 1989). In large blocks sanitary conditions as well as cockroach population size are highly variable between flats (Marsh and Berthold, 1986). In addition, cockroaches are omnivorous, and their feeding habits are such that they are in contact with many kinds of stored food used by people, as well as with different kinds of biological waste or detritus, garbage and sewage, dead insects, feces, etc. (Cochran, 1982).

In Bangladesh, cockroaches are considered as one of the most important pests of our household and business establishments. The mere presence of cockroaches is a sign of unhygienic condition. In developing countries people suffer from food-borne bacterial illnesses, especially from Bacteria in the genera *Salmonella* (food poisoning), *Staphylococcus, Streptococus, Coliform, Bacillus* and *Clostridium,* the bacteria *Escherichia coli* (Diarrhea) and *Shigella dysenteriae* (Dysentery), the protozoan-caused parasitic toxoplasmosis. Unhygienic food handling results in food contaminated by pathogens. One possible source of food contaminations could be dissemination of the pathogens to foods and/or utensils of catering centers through small animals such as cockroaches that live closely with human and animals. Various investigations around the world revealed that cockroaches living close to human dwellings were important carriers of etiologic agents belonging to all groups of potential pathogens: viral, bacterial, protozoan and helminthes (Burgess and Chetwyn, 1981; Agbodaze and Owusu, 1989; Fotedar *et al;* 1991a; Cloarec *et al.,* 1992; Pai *et al.,* 2003ab).

Almost all cockroaches isolated from catering centre residential areas carried medically important microorganisms. A study from South Africa showed that pathogenic strains isolated from cockroaches were indistinguishable from those colonizing infants (Cotton et al., 2000) and some of the isolates were resistant to antimicrobial drugs (Fathpour *et al.,* 2003). Various food-borne pathogens were isolated from cockroaches collected from kitchens in Ghana (Agbodaze and Owusu, 1989) and in Nigeria (Umunnabuike and Irokamulo, 1986). In Bangladesh, *Salmonella, Shigella, S. aureus, B. cereus,* and *E. coli* were isolated from cockroaches (Paul *et al.,* 1992). Survival of pathogens in experimentally infected cockroaches was reported by (Fotedar *et al.,* 1993; and Imamura *et al.,* 2003).

Antimicrobial resistance is an increasingly global problem, and emerging antimicrobial resistance has become a public health issue worldwide (Kaye *et al*., 2004). A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in humans or veterinary medicine and in food-animal production (Bager and Helmuth, 2001; Anderson *et al*., 2003; Schroeder *et al*., 2004). These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens (Schroeder *et al*., 2004). Based on the above background, the necessity of the present study is developed.

1.1. Aims and objectives

The main objective of this study is to investigate the presence of *Staphylococcus* spp. in cockroach's and antimicrobial sensitivity testing of the isolates to determine resistance pattern by Disc Diffusion method. The following specific objectives were fulfilled by the present research

- To estimate prevalence of *Staphylococcus* in the external washings and gut isolates of cockroaches .
- To determine antimicrobial resistance pattern of isolated *Staphylococcus* from the isolates of cockroaches.

1.2. Significance of the study

Staphylococcus spp. is recognized as a major cause of food poisoning, wound infections, skin infections, infections of internal organ which are closely associated with the consumption of contaminated food. The study will help to know the prevalence of *Staphylococcus* spp. in cockroaches. On the otherhand, an attempt has been taken to analysis of antibiotic resistant in *Staphylococcus* spp isolated and identified from cockroaches. The identification of antimicrobial resistant *Staphylococcus* will help for appropriate therapy and provide information on the auxiliary emergence of drug resistance.

Chapter-2: Review of Literatures

2.1 Characteristics, taxonomy and nomenclature of Staphylococcus

Staphylococcus aureus is a normal and ubiquitous Gram-positive coccal inhabitant of skin and mucous membranes of a wide range of animals, including poultry and waterfowl (Skeeles, 1991). *Staphylococcus aureus* is a facultative anaerobic Gram-positive coccus; it is non-motile and catalase and coagulase positive. Cells are spherical single or paired cocci, or form grape-like clusters (*staphylo* means grape in greek). The staphylococcal cell wall is resistant to lysozyme and sensitive to lysostaphin, which specifically cleaves the pentaglycin bridges of *Staphylococcus* spp. Some *S. aureus* strains are able to produce staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisonings. Unlike *C. perfringens*, *C. botulinum*, and B. *cereus*, it does not form spores. Thus, *S. aureus* contamination can be readily avoided by heat treatment of food. Nevertheless, it remains a major cause of FBD because it can contaminate food products during preparation and processing. *Staphylococcus aureus* is indeed found in the nostrils, and on the skin and hair of warm-blooded animals. Up to 30-50% of the human population is carriers. *Staphylococcus aureus* is able to grow in a wide range of temperatures (7° to 48.5°C with an optimum of 30 to 37°C (Schmitt *et al.,* 1990), pH (4.2 to 9.3, with an optimum of 7 to 7.5; (Bergdoll, 1989) and sodium chloride concentrations (up to 15% NaCl). These characteristics enable *S. aureus* to grow in a wide variety of foods. This, plus their ecological niche, can easily explain their incidence in foodstuffs that require manipulation during processing, including fermented food products, such as cheeses. Risk assessment in foodstuffs relies on classical microbial detection and quantification of coagulase positive staphylococci on a selective Baird-Parker medium. Sensitivity of these routine tests is around 10^2cfu/g for solid foodstuffs and 10cfu/g for liquid samples. The different media used for the detection and quantification of *S. aureus* have been reviewed by Baird and Lee, (1995). In many countries, low degree contaminations by *S. aureus* are tolerated in most foodstuffs (up to 10^3 cfu/g in raw milk cheeses, in France), as they are not considered a risk for public health.

Staphylococcus aureus strains can be classified into biotypes according to their human or animal origin. Devriese (1984) developed a biotype schema, including six different biotypes (human, non-β-hemolytic human, avian, bovine, ovine, and nonspecific), based on biochemical characteristics.

2.2 Staphylococcosis

S. aureus is one of the most prominent human pathogens and is responsible for diverse infections worldwide (Lowy, F. D. 1998). *Staphylococcus aureus* is a major cause of bacteremia, and *S. aureus bacteremia* is associated with high morbidity and mortality, compared with bacteremia caused by other pathogens. The burden of *S. aureus* bacteremia, particularly methicillin-resistant *S. aureus* bacteremia, in terms of cost and resource use is high. The risk of infective endocarditis and of seeding to other metastatic foci increases the risk of mortality and raises the stakes for early, appropriate treatment. The incidence of *S. aureus* bacteremia and its complications has increased sharply in recent years because of the increased frequency of invasive procedures, increased numbers of immune compromised patients, and increased resistance of *S. aureus* strains to available antibiotics. This changing epidemiology of *S. aureus* bacteremia, in combination with the inherent virulence of the pathogen, is driving an urgent need for improved strategies and better antibiotics to prevent and treat *S. aureus* bacteremia and its complications (Christoph K. Naber, 2007)

Staphylococcus aureus is a leading cause of human infections worldwide. The pathogen causes a variety of diseases including impetigo, cellulitis, food poisoning, toxic shock syndrome, necrotizing pneumonia, endocarditis, and sepsis. Risk of *S. aureus* infection is increased during hospitalization, after surgery or dialysis, and in patients with indwelling percutaneous medical devices and/or catheters (Lowy, FD.1998). *S. aureus* is one of the only causes of endocarditis instructurally normal heart valves (Lesse and Mylotte, 2006). Notably, there is an alarming increase in the incidence of community-acquired (CA) *S. aureus* infections in seemingly healthy individuals, a problem which underscores the need to better understand virulence mechanisms (Said-Salim et al., 2003). *Staphylococcus aureus* (*S. aureus*) infections are a major problem in rabbits (Corpa et al., 2009). These infections are commonly presented in the form of pododermatitis, subcutaneous abscesses and mastitis in all rabbit age groups. Sporadically, purulent inflammatory lesions are observed in internal organs, predominantly in lungs, liver and uterus (Hermans *et al.*, 2003; Vancraeynest *et al.*, 2004). *S. aureus* produces a wide spectrum of virulence factors and many of the diseases caused by this bacterium in livestock, including rabbits, could be attributed to the virulence factors the bacteria produce, which include haemolysins, enterotoxins, toxic shock syndrome toxin-1 (Tenover and Gaynes, 2000; Vancraeynest *et al.*, 2006; Meulemans *et al*., 2011). The gross and microscopic lesions observed in birds dying from staphylococcosis are necrosis, vascular congestion and inflammation in the liver, kidneys, spleen and lungs.Plantar abscesses, arthritis, tenosynovitis and osteomyelitis, myocarditis, pericarditis and thromboendocarditis have also been reported (Arp *et al.,* 1983; Bergmann *et al.,* 1988; Cheville *et al.,* 1988; Emslie & Nade, 1985; T-W-Fiennes, 1982; Skeeles, 1991; Wobeser, 1981; Wobeser & Kost, 1992).

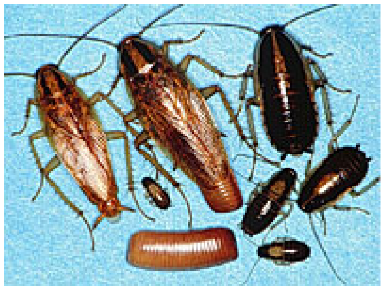
2.3 Diseases Spread By Cockroaches

Cockroaches are persistent and troublesome pests of homes, restaurants, hospitals, warehouses, offices, and other structures with food handling areas. These insects contaminate food and utensils, destroy fabric and paper products, and impart stain and odor to surfaces they contact.

Cockroaches are universally loathed. One of the primary reasons is because they are associated with any place where there are biological waste products such as sewers, septic tanks, garbage cans, chicken houses, and animal cages. Their attraction to human and animal feces, rotting food, secretions from corpses, sputum, pus, and the like gives them a well-earned "disgust factor" among the general public. All these moist, organic habitats contain staggering amounts of bacteria, protozoa, amoebae, fungi, and other microbial material (Bell *et al.,* 2007).

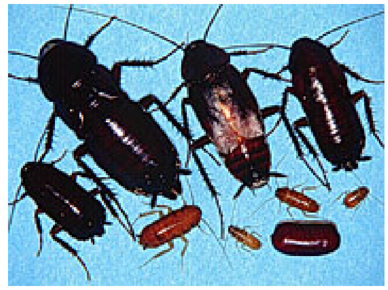
Cockroaches belong to the insect order Dictyoptera. Young and immature cockroaches resemble adults, that is, they undergo gradual metamorphosis. Adults of male species have wings, although many species do not fly. Although there are 70 described species of cockroaches in the United States, and over 3,500 worldwide, only a few are major pests. Occasionally species that usually occur outdoors invade buildings.

Microbes are an essential influence in the nutrition, ecology, and evolution of all cockroaches. The main source of nourishment for cockroaches in mines and sewers, for example, is human feces, which can be 80% bacterial. German cockroaches have been observed feeding on mouth secretions of corpses riddled with lung disease; these secretions were almost 100% infectious bacteria. (Bell *et al.,* 2007).



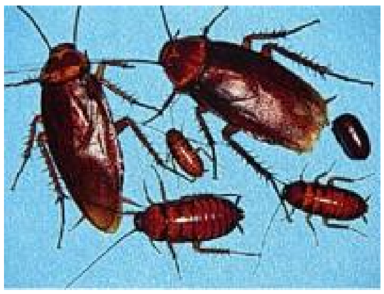
German cockroach

(*Blatella germanica*)



Oriental cockroach

(*Blatta orientalis*)



American cockroach

(*Periplaneta americans*)

Asian Cockroach

(*Blattella asahinai)*

Smokybrown cockroach

(*Periplaneta fuliginosa*)

Brownbanded cockroach

(*Supella longipalpa*)

Fig-2.1: Different types of cockroach

The makeup of the microbial population of the American cockroach gut, long the subject of numerous investigations, has been shown to include a variety of microorganisms, bacteria, archeans, protozoa, and nematods. Cockroaches, especially species that come in contact with feces like German cockroaches may transmit bacteria responsible for food poisoning. Cockroaches act as mechanical vector in transmitting *Salmonella, Shigella*, and *Cryptosporidium parvum* bacteria that cause diarrheal diseases. Antibiotic resistant strain of

Klebsiella pneumoniae bacteria that cause pneumonia has been found in patients and cockroaches in a New Delhi hospital. In addition, evidence suggests that cockroaches spread typhoid, dysentery, and leprosy organisms (Marer *el al.,* 2006).

2.3.1 Bacteria Carried By Cockroaches

At least 32 species of bacteria have been isolated from cockroaches in domestic environments. These include:

- **Aeromonas spp.,** cause wound and other infections, diarrhea.
- ***Alcaligenes faecalis*,** causes of gastroenteritis, urinary tract infections.
- ***Bacillus cerreus*,** causes food poisoning.
- ***Bacillus subtilis*,** causes conjunctivitis.
- **Campylobacter jejuni,** causes enteritis.
- **Clostridium perfringens,** causes food poisoning, gas gangrene.
- ***Enterobacter* spp.,** cause bacteremia (temporary presence of bacteria in the blood, which is commonly followed by the development of various infections including abscesses).
- ***Enterococcus* spp.,** cause urinary tract and wound infections.
- ***Escherichia coli.,*** cause diarrhea, wound infections.
- **Klebsiella spp.,** cause pneumonia and urinary tract infections.
- ***Mycobacterium leprae*,** causes leprosy.
- ***Morganella morganii*,** causes wound infections.
- ***Nocardia* spp.** causes actinomycetoma (chronic infection of the skin and underlying tissues).
- ***Proteus rettgeri*,** causes wound infections.
- ***Proteus vulgaris*,** causes wound infections.
- ***Proteus mirabilis*** causes wound infections, gastroenteritis.
- **Pseudomonas spp.,** cause respiratory infections, gastroenteritis.
- ***Salmonella* spp.,** cause gastroenteritis, food poisoning.
- ***Salmonella typhi*,** causes typhoid.
- ***Salmonella pyogenes*,** causes pneumonia.
- **Serratia spp.,** cause food poisoning.
- ***Shigella dysenteriae*,** causes dysentery.

- ***Sphyngobacterium* spp.** causes sepsis (presence in the blood or other tissues of pathogenic microorganisms or their toxins).
- **Staphylococcus aureus,** causes wound infections, skin infections, infections of internal organ.
- **Staphylococcus epidermalis,** causes wound infections.
- **Streptococcus faecalis** and other species, cause pneumonia.
- ***Yersinia pestis*** (isolated from oriental cockroach), causes plague.

2.3.2 Worms, Protozoa, Fungi, and Viruses Carried By Cockroaches

Cockroaches also have been found harboring eggs of seven species of helminths (hookworm, giant human roundworm, pinworm, tapeworm, and whipworm); at least 17 fungal species, three protozoan species, and two strains of polymyelitis virus. Australian, American, and Madeira cockroaches become infected with the protozoan ***Toxoplasma gondii***, the causative agent of toxoplasmosis, after feeding on feces of infected cats.

This suggests the possibility of cockroach involvement in the maintenance and dissemination of this parasite, which infects humans and other animals (Mullen and Durden, 2002).

At least eleven proteins isolated from German and American cockroaches can cause allergic reactions and contribute to asthma in humans. The allergens are heat-stable and persistent in the environment even after the insect death. The cockroach mite (*Pimeliaphilus cunliffei*) is a parasite of cockroaches. It feeds on live individuals and has been linked to bites of humans living in households with cockroach infestations.

2.4 Antimicrobial resistance

One of the most important problems facing global public health today is antimicrobial resistance. The problem is most horrible in developing countries, where the bacterial infections causing human disease are also those in which emerging antibiotic resistance is most evident (Shears 2000; Fathpour *et al.,* 2003; Kalantar *et al.,* 2008). Antibiotic resistance in microorganisms is either genetically inherent or the result of the microorganism being exposed to antibiotic. Most of the antibiotic resistance has emerged as a result of mutation or through transfer of genetic material between microorganisms. A broad variety of biochemical and physiological mechanisms are responsible for the development of resistance. Recent studies of almost 400 different bacteria have demonstrated about 20,000 possible resistance genes (r genes) (Davies *et al.,* 2010). In many developing countries the use of antimicrobial drugs for treating people and animals is unregulated; antibiotics can be purchased in pharmacies, general stores, and even market stalls. In the Rajbari district of Bangladesh, a survey of rural medical practitioners (barefoot doctors) with an average of 11 years' experience showed that they each saw on average 380 patients per month and prescribed antibiotics to 60% of these patients on the basis of symptoms alone (Mamun, 1991).

2.4.1 Mechanism of action of antimicrobial resistance:

2.4.1.1 Natural Resistance

Antibiotic usage by human and releasing to the environment can promote antibiotic resistance in every place. Antibiotics are used as growth support for animal, therapeutic agent for humans, aquacultures, pets and pest control in agriculture, biocides in toiletries, hand care, culture sterility, cloning and industry (Davies *et al*., 2010). Differences in environmental factors could be responsible in existence of numerous number and sorts of resistance (Davies *et al*., 2010). Intrinsic resistance is the survival genes in bacterial genomes that could create a resistance phenotype (Davies *et al*., 2010). Genetic studies showed that wastewater and plants are reservoirs of antibiotic resistance (Davies *et al.,* 2010).

2.4.1.2 Mutation

Genetic mutation is the primary cause of antibiotic resistance. Antibiotic resistant is created from different ways, such as antibiotic usage both in medical and veterinary medicine that leads to distribution of bacterial resistance genes through other bacteria (Mathew *et al*., 2007). Resistance in bacteria could be inherent. This can happen by mutation or acquirement of new DNA. Mutation is spontaneous and transfer by plasmids or bacteriophages (Hawkey, 1998).

2.4.1.2.1 Examples of Antibiotic Resistance through Mutation

Some of bacteria have the ability to produce specific antibodies that are resistant to an extended Spectrum β-lactamase (ESBLs). The common place for these bacteria is gut. These bacteria are expanded among patients and in hospitals. These groups of bacteria can then be transferred to other people by unwashed hands specially after using toilet (Davies *et al*., 2010). The genes of β-lactamase enzymes are mainly global in circulation; random mutation of the genes encoding and the enzymes have increase to customized catalysts with ever more complete spectra of resistance. Another general family of DNA- binding proteins gyrase inhibitors is answerable for low levels of Quinolone resistance (Davies *et al*., 2010).

2.4.1.2 Gene Transfer

Genes can be transferred between bacteria in a horizontal way through conjugation, transduction and transformation. Transfer of genes can happen in the intestinal tracts of animals and human. Thus genes of antibiotic resistance can be shared. Most of antibiotic resistance genes reside on plasmids with self replicating circular pieces of DNA that make the transfer easier. If a bacteria have several resistance genes, it is called multi-resistant or a superbug or super bacterium. The name superbug refers to microbes with high levels of resistance to antibiotic. Some super resistant strains have high virulence and enhanced transmissibility. Certain antibiotic are associated with higher levels of development of superbugs (Davies *et al*., 2010). It is proposed that soil microbes are reservoirs for antibiotic resistance genes that can transfer to other microbes (Mathew et *al*., 2007). Stress increases the prevalence of resistance and the skills of bacteria to obtain these genes that may be absorb by the similar genetic elements in the bacterium that receive the resistant gene (Mathew *et al*., 2007). The efflux systems can force offending compounds, like as antibiotics out of the cell and can produce a wide range of resistance ((Mathew *et al*., 2007). In some cases, the resistance genes can also import unknown advantages beyond those associated with the advantages selected under antibiotic use (Mc Dermott *et al*., 2002).

2.5 Development of Resistance

Antibiotic resistance is a global problem in public health and is growing around the world. Antibiotics have been used for 70 years but during the last decade some treatments have become ineffective and this may lead to spread of some infections in the future. Antimicrobial resistance (AMR) is created by use of antibiotics in a wrong way and develops when a microorganism have mutated or acquired inappropriate use of antibiotics in human and veterinary medicine leads to higher frequencies of AMR (Mc Dermott *et al.,* 2002). Antibiotics are often used in animals. Transfer to human's food of these antibiotics can affect the safety of the meat, milk, and eggs produced and can be the source of superbugs. The resistant bacteria in animals can transfer to humans by three pathways, consumption of meat or other food, direct contact with animals or through the environment. In many developing countries the use of antimicrobial drugs for treating people and animals is unregulated; antibiotics can be purchased in pharmacies, general stores, and even market stalls. In the

Rajbari district of Bangladesh, a survey of rural medical practitioners (barefoot doctors) with an average of 11 years' experience showed that they each saw on average 380 patients per month and prescribed antibiotics to 60% of these patients on the basis of symptoms alone (Mamun, 1991).

2.5.1The Biological Side of Resistance

Antibiotics were being discovered and developed for clinical use, some of the particular traits of bacteria were also being discovered, mainly their ability to exchange genetic information (Amabile-Cuevas, 2003). As the antibiotic era evolved, so did our understanding of bacteria, and bacteria 4 C.F. Ama bile-Cuevas themselves. A striking example of this simultaneous evolution is provided by quinolones and its associated resistance. Introduced in the 1960s, starting with nalidixic acid, quinolones had an explosive growth with the advent of fluorinated derivatives in the 1980s. Resistance emerged but was supposed to be restrained to vertical inheritance (Fuchs *et al.,* 1996), and a mini-review paper in the early 1990s posed the question of the – so far – missing plasmid-mediated quinolone resistance (Courvalin, 1990). Shortly before, a remarkable stress defense mechanism of several gram-negative bacteria, the mar regulon, was discovered among fluoroquinolone-resistant clinical isolates (Hooper *et al.,* 1989). And shortly after, the first of a series of plasmid-borne fluoroquinolone genes was reported, possibly evolved from microcin-resistant traits (Tran and Jacoby, 2002), and soon followed by a variety of similar genes. And then, an aminoglycoside-modifying enzyme evolved to modify also a fluoroquinolone (Robicsek *et al.,* 2006), an entirely different molecule, both chemically and pharmacologically. Meanwhile, in the gram-positive side, where plasmidmediated quinolone resistance is yet to be found, the supposed restriction to vertical inheritance was also bypassed by transformation, allowing resistant alleles of target genes to be mobilized among different streptococcal species (Balsalobre *et al.,* 2003). And this is just an example. In addition to the quinolone story, we have learned during the past 20 years or so about the many ways bacteria can successfully face the threat of antibiotics. Some of these include (a) global responses to environmental stress, which include the mar regulon earlier mentioned, and the somehow related oxidative-stress response system, the soxRS regulon of Escherichia coli and Salmonella enterica (Demple and Ama bile-Cuevas, 2003), along with several other efflux-mediated resistance mechanisms (Davin-Regli and Page`s , 2007), but also including the SOS response (Miller *et al.,* 2004), which can, additionally, promote the horizontal dissemination of resistance genes (Beaber *et al.,* 2003); (b) biofilm growth and its conferred resistance, or tolerance, the mechanism of which is still a matter of debate (Gilbert *et al.,* 2007), but that is clearly causing antibiotic treatment failure. Also, biofilms are playgrounds for bacteria in terms of exchanging and ‘‘concentrating’’ canonical resistance traits (Delissalde and Ama bile-Cuevas, 2004); (c) plasmid-antibiotic resistance interdependency, in a way similar to the toxin–antitoxin systems that prevent the emergence of plasmid-less cells within a resistant bacteria population (Heinemann and Silby, 2003), and that is but one cause of the overall failure of antibiotic cycling strategies within hospitals; (d) resistance gene mobility, mainly mediated by plasmids, which has been known for more than 60 years (Amabile-Cuevas and Chicurel, 1992) but whose components we are still trying to understand: integrons and gene cassettes, transposons including insertion sequences and conjugative transposons, conjugative and mobilizable plasmids, etc. (Amabile- Cuevas, 1993). All these features, and perhaps many more that we are yet to discover, make resistance very resistant to elimination and even control. Additionally, antibiotic usage is not the only selective pressure that we are applying to foster antibiotic resistance: disinfectants (Aiello and Larson, 2003), 1 Global Perspectives of Antibiotic Resistance 5 environmental pollutants (Jimenez-Arribas *et al.,* 2001), etc. can keep the prevalence of resistance high, even if we manage to restrain the abuse of antibiotics.

2.5.2 The Pharmacological Side of Resistance

Two pharmacological issues have particular relevance to the resistance problem: (a) spectrum and (b) compliance. Wide-spectrum antibiotics do have an important role in fighting infection, either that caused by several different bacterial species or those for which assessing the etiology is too difficult or takes too much time. However, wide spectrum has been an obvious goal in the R&D antibiotics, as it ensures also a wide variety of clinical uses and, of course, of sales. Wide spectrum has also been presented to the medical community as a general advantage so that the physician need not worry about the etiology of an infectious disease to start treatment. Of course, the likelihood of this strategy to succeed in the short term and the individual patient is high. But this shotgun notion contributes to resistance as it applies selective pressure, not only upon the etiological agent of the infectious episode but also upon a larger fraction of the patient’s microbiota. Some reports indicate that, although worldwide use of antibiotics is receding, the use of wide-spectrum ones is dramatically increasing. Along with increasing resistance trends, marketing efforts are aimed at positioning newer fluoroquinolones as choice drugs against lower respiratory tract infections, instead of aminopenicillins and macrolides, and even oral, third generation cephalosporins against diseases as minor as pharyngotonsillitis. It is of course not surprising to see among community-acquired uropathogens up to one-third of them highly resistant to fluoroquinolones, and even a high prevalence of ESBL enzymes, previously confined to hospital settings (Casellas and Quinteros, 2007).

2.5.3 The Clinical Consequences of Resistance

Resistant germs are more difficult to get rid off and that complications and deaths resulting from infections caused by them will only increase in time. Very few really new antibiotics will be developed in the short- and mid-term, and perhaps we already have as many antibiotics as possible, judging from recent genomic evidences (Becker *et al.*, 2006). From a global point of view, this means that infections will impose a growing toll on humans, despite the dramatic reduction that we accomplished during the first 80 years of the twentieth century. But it will be different between regions. In the first paragraph of this chapter, I mentioned the alarm caused by rising MRSA infections and deaths in the United States; but in ‘‘developing’’ countries, MRSA is not as big a problem as multi-resistant salmonellosis, shigellosis, and tuberculosis in the community setting and enteric bacteria and Pseudomonas aeruginosa infections in hospitals. Some may think that resistance is affecting equally the developed and developing countries, if only through different bugs. However, developing countries do not have pharmaceutical research of their own, so we are dependent on what developed countries do develop; and their worries are different from ours. So while new anti-staphylococcal drugs are actually being deployed (linezolid, daptomycin, newer glycopeptides), drugs that can be used against MDR- and XDR-TB, multi-resistant Shigella and Salmonella strains, or multi-resistant enteric bacteria causing nosocomial infections much more often in our deficient hospitals are not being explored.

2.6 Global trends in resistance pattern

Antimicrobial resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions, poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance (Byarugaba, 2004), misdiagnosis, counterfeit drugs and lack of education in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases (Walia, 2006).

2.7 Reason for antibiotic resistance in developing countries:

2.7.1 Misuse of Antibiotics by Physicians in Clinical Practice

Antibiotic use provides selective pressure favoring resistant bacterial strains; inappropriate use increases the risk for selection and dissemination of antibiotic-resistant bacteria, which are placed at a competitive advantage. Therefore, one would expect that drugs more commonly affected by bacterial resistance in developing countries are generally inexpensive and popular broad-spectrum agents (Murray *et al.,* 1985; Sack *et al.,* 1997; Rahal *et al.,* 1997; Hoge *et al.,* 1998; And Calva *et al.,* 1996) Clinical misuse of antibiotics may be more common among private practitioners than among public health personnel. Private practitioners charge higher fees, the demand for antibiotics seen in private patients is higher, and more drugs are available in private clinics than in public hospitals (Muhuri *et al.,* 1996; Cash, 1996; Lee and Henry, 1989).

2.7.2Misuse of Antibiotics by Unskilled Practitioners

In many developing countries, well-trained health personnel are scarce and cannot serve the entire population, especially in rural areas. Community health workers and others with minimal training treat minor ailments (Pearson, 1995). The qualifications and training of community health workers, as well as the quality of care they provide, vary from country to country. Unskilled personnel are less aware of the deleterious effects of inappropriate antibiotic use. For example, pharmacy technicians in Thailand prescribed rifampicin for urethritis and tetracycline for young children (Thamlikitkul, 1988).

2.7.3Misuse of Antibiotics by the Public

In most developing countries, antibiotics can be purchased without prescription, even when the practice is not legal. In many African, Asian, and Latin American countries, antibiotics are readily available on demand from hospitals, pharmacies; patent medicine stalls (drugstores), roadside stalls, and hawkers (Dua *et al.,* 1994; Kafle *et al.,* 1992). In rural Bangladesh, for example, 95% of drugs consumed for 1 month by more than 2,000 study participants came from local pharmacies; only 8% were prescribed by physicians (Hossain *et al.,* 1982).

2.7.4 Poor Quality of Antibiotics

2.7.4.1 Lack of Quality Compliance and Monitoring

Besides the risk for therapeutic failure, degradation products or adulterants in poor quality antibiotics can produce subinhibitory concentrations in vivo, which increase the selection of resistant strains. Drugs that do not comply with minimum standards are illegal in all countries. However, the quality of many antibiotics and other drugs in developing countries is often below standards in the formulary. In Nigeria for example, substandard ampicillin, ampicillin/cloxacillin, tetracycline, and oxytetracycline capsules have been detected (Okeke and Lamikanra 1995; Esezobo and Offiong 1986; Agom *et al.,* 1990 and Taylor *et al.,* 1995). In many cases, therapeutic failure is the only indication of substandard drugs. Analytic laboratories to detect substandard drugs are uncommon, and when they exist, health workers, distributors, and consumers are often unaware of them.

2.7.4.2 Degraded Antibiotics

The shelf lives of drugs developed and marketed in temperate countries are determined by storage temperatures. During distribution in tropical countries, conditions of transport and storage are poorly controlled, and the drugs may be degraded. Ballereau *et al.,*1997 recorded temperatures of 26°C to 40°C and 30% and 90% humidity in Guinea-Bissau during a 2-year period (temperatures of greater than 25°C can degrade antibiotics). Many antibiotics, being heat- and moisture-labile, are particularly vulnerable. Of seven drugs that lost 10% or more of their active constituents when stored in pharmacies in Guinea-Bissau for 2 years, six were antimicrobial drugs (Ballereau *et al.,* 1997) Drug consignments are exposed to such adverse conditions during shipment (Hogerzeil *et al.,* 1992).

2.7.4.3 Expired Antibiotics

Some pharmacologically active drugs produced in industrialized countries have expired when distributed in developing countries. They were shipped at the end of the drugs. Shelf lives or their clearance and distribution after transcontinental shipment were delayed. Expired drugs may receive new labels, be dumped without a label change, or be donated rather than sold (Hogerzeil *et al.,* 1992; Gustafsson, 1981 and Ali *et al.,* 1988). Tax deductions and the cost of liquidation are incentives for donating expired or near-expired drugs. Effective enforcement of the World Health Organization (WHO) guidelines on drug donations may curtail such practices (Berckmans *et al.,* 1996).

2.7.4.4 Counterfeit Drugs

Some drugs sold in developing countries do not contain the concentration of active substances stated on their labels, even at the time of manufacture. These counterfeit drugs flourish, despite efforts of local regulatory agencies to stop their production and distribution (Adjepon-Yamaoah, 1980; Land, 1992 and McGregor, 1997). Approximately 65% of the 751 instances of counterfeit pharmaceuticals reported to WHO or to Interpol from 28 countries in the past 15 years were produced in developing countries (McGregor, 1997).

2.7.4.5 Adulterated Drugs

Herbal preparations in developing countries are often adulterated with orthodox medicaments. For example, in one study, 24% of Chinese herbal preparations marketed in Taiwan contained one or more of such adulterants (Huang *et al.,* 1997). Although the adulteration of such products with antibiotics has not been reported, such practices may be common (Michel, 1985). A Nigerian traditional healer, for example, admitted to augmenting herbal preparations with tetracycline from commercially available capsules (Ogungbamilaand, 1993).

2.7.5 Crowding and Unhygienic Conditions

Residents of developing countries often carry antibiotic-resistant fecal commensal organisms (Calva *et al.,* 1996; Lamikanra *et al.,* 1989). Visitors to developing countries passively acquire antibiotic-resistant gut Escherichia coli, even if they are not taking prophylactic antibiotics, which suggests that they encounter a reservoir of antibiotic-resistant strains during travel (Murray *et al.,* 1990). Apparently healthy people indeveloping countries carry potentially pathogenic, antibiotic-resistant organisms asymptomatically (Woolfson *et al.,* 1997).

2.7.6 Inadequate Hospital Infection Control Practices

Infection control practices in many hospitals in developing countries are rudimentary an often compromised by economic shortfalls and opposing traditional values (Meers, 1988). The resulting nidus of nosocomial pathogens and resistant organisms may be disseminated to the outside\ community. Improper disposal of hospital waste accentuates such spread. Untreated hospital waste in Uganda was often dumped into public sewers or thrown into rubbish heaps ravaged by scavengers (Okello *et al.,* 1997).

2.7.7 Inadequate Surveillance

Information from routine susceptibility testing of bacterial isolates and surveillance of antibiotic resistance, which provides information on resistance trends, including emerging antibiotic resistance, is essential for clinical practice and for rational policies against antibiotic resistance. Bacterial infections are often treated after they become life-threatening, which encourages empirical selection of broadspectrum antibiotics (Shann, 1995). The antibiotic susceptibility pattern of bacterial isolates in much of the developing world is unknown, and little guides empirical prescribing. Susceptibility testing cannot be done readily because equipment, personnel, and consumables are scarce and expensive (Yang *et al.,* 1993 and Brown, 1996).

2.7.8 Economic and Political Factors

Lack of resources hampers implementation of most strategies against antibiotic resistance. Statistics from the World Bank show that developing countries spent $41 per person on health in 1990, compared with the $1,500 per person spent by industrialized countries. Disease prevalence as measured by disabilityadjusted life years and by communicable disease in particular is much greater in developing than in industrialized countries (Korte *et al.,* 1991; Shah ,1993; Summerfield, 1993; Murray and Lopez, 1997). As a result of such gross underfunding, the drug supply is chronically inadequate or at best erratic in health facilities in many countries, including Nigeria (Dua *et al.,* 1997).

2.7.9 Public Health importance:

S*taphylococcus aureus* (*S. aureus*) is an important pathogen of domestic ruminants and man (Bukharie *et al.,* 2001; Grundmann *et al.,* 2006; Fitzgerald, 2012), and is regarded as one of the most versatile and devastating zoonotic pathogen responsible for causing widespread outbreaks of serious infection, food poisoning, colonization, animal mastitis and pneumonia affecting man and animals (Fthenakis, 1998; Simoons-Smit *et al.,* 2000; Kesah *et al.,* 2003; Pengov and Ceru, 2003; Eftekhar and Dadaei, 2011; Ateba Ngoa *et al.,* 2012; Dhama *et al.,* 2013). Cockroaches are among the varieties of medically important insects found in urban and rural environments that cause serious public health problems (Sramova *et al.*, 1992;

Graczyk *et al*., 2001) In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, food borne diseases still dominate as the most important public health problem in most countries (Dominguez *et al.,* 2002). Many foods, particularly those of animal origin, have been identified as vehicles for transmission of these pathogens to human beings and spreading them to the processing and kitchen environment (Uyttendaele *et al.,* 1998). In developed countries food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems (Jordan *et al.,* 2006). Antimicrobial residues exceeding the threshold level have been detected in animal and poultry products in Bangladesh (Chowdhury, 2012; Hossain, 2012; Mahmud, 2013).

Chapter-3: Materials and Methods

3.1. Study area

Three household and four restaurants of Chittagong City Corporation (CCC) were randomly selected where large number of people had their feed every day.

3.2. Study period

The study was conducted for a period of six months from July to December, 2014.

3.3. Collection and identification of cockroaches

The households had at least six members and the restaurants represented medium level eating centers which served about 200 customers per day were selected for the present study. Cockroaches were collected using sterile screw-capped 250-ml jars and sterile hand-gloves (Paul *et al.,* 1992). Only cockroaches caught whole and alive were considered in the study. Identification of cockroaches was performed in accordance with Burgess, (1993).

Fig-3.1: Identification of Cockroaches

3.4. Processing of cockroaches for isolation of pathogens

The collected cockroaches were brought to the laboratory and killed in a sterile jar using chloroform soaked cotton. The external body surface was washed by vortexing in 5-ml sterile physiological saline for two minutes, and the wash was taken as external body homogenate sample. After external body washing, the cockroaches were soaked in 90% ethanol for five minutes to decontaminate their external surfaces and were dried. They were then re-washed with sterile saline to remove traces of ethanol, and the alimentary tract was aseptically dissected out using autoclave-sterilized entomological dissecting needles. The instruments were dipped in ethanol and flamed between dissections. The excised gut was then homogenized in 5-ml of sterile normal saline water. A total of (50) specimens consisting of (25) external body surface and (25) gut homogenates of the cockroaches were analyzed. For primary enrichment, 1-ml of each homogenate was inoculated separately into 9-ml of buffered peptone water (BPW) (OXOID, Basingstoke, UK) and incubated at 37°C for 18-24 h.

Fig-3.2: Killing of Cockroaches

Fig-3.3: Dissecting of Cockroaches

3.5. Isolation and identification of pathogens

For the isolation of *Staphylococcus* spp. *g*rowth from BPW was heavily plated on Mannitol Salt Agar (MSA) (OXOID, Basingstoke, UK) and incubated at 37°C for 48 h. Mannitol fermenting colonies were further characterized by microscopic examinations.

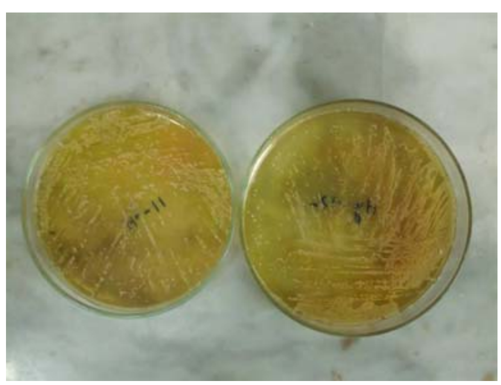
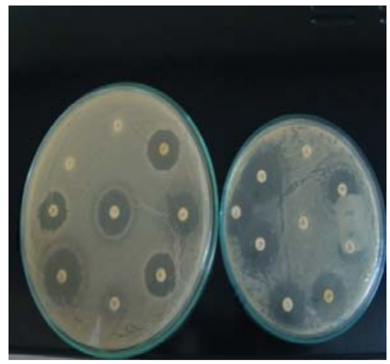


Fig-3.4: Growth of *Staphylococcus* on Mannital Salt agar

3.6. In-vitro drug susceptibility testing

Susceptibility testing was done on Mueller-Hinton agar plates following the standardized disk diffusion technique (Bauer *et al.,* 1966) with (OXOID, Basingstoke, UK) drug discs: cephalothin (Cep), (30µg); kanamycin (Kan), (30µg); clindamycin (Cli),(2µg); oxacillin (Oxa), (1µg); erythromycin, (15µg); penicillin, (P), (10unit); *Staphylococcus* spp are sensitive to all the drugs used in this study, were routinely tested. Interpretation of readings as sensitive, intermediate and resistant was made according to a chart (Jorgenson *et al.,* 1999).

Inserting antibiotic disk



Zone of inhibition

Figure-3.5: Antibiogram of Staphylococcus spp.

Table-3.1: Panel of antibiotics used, their concentrations and zone diameter interpretative standards was determined based on CLSI, (2007)

Group of Antimicrobial	Antimicrobial	Disk contents	Zone diameter, nearest whole mm		
			R	**I**	**S**
Aminoglycosides	Kanamycin	30 µg	≤ 13	14-17	≥ 18
Macrolides	Erythromycin	15 µg	≤ 13	14-22	≥ 23
Cephems	Cephalothin	30 µg	≤ 14	15-17	≥ 18
Lincosamides	Clindamycin	2 µg	≤ 14	15-20	≥ 21
Penicillins	Penicillin	10 unit	≤ 28	-	≥ 29
Penicillins	Oxacillin	1 µg	≤ 10	11-12	≥ 13

3.7 Data Analysis

Field and Laboratory data were stored and then cleaned in the MS excel-2007 programme before exporting to STATA/IC-13.0 for analysis. Descriptive analysis was performed to know the frequency & distribution of *Staphylococcus* and antibiotic resistance pattern.

Chapter-4: Results

Table 4.1: Prevalence of *Staphylococcus* in different samples and sampling sites

Variables	Categories	positive (%)	Chi2 -value	p-value
External washing	Household	7 (46.67)	0.1082	0.742
	Restaurant	4 (40.00)		
Gut contents	Household	10 (66.67)	1.7316	o.188
	Restaurant	4 (40.00)		

Table-4.1 shows the prevalence of *staphylococcus* spp. in the external and gut isolates of cockroach collected from households and restaurants. The highest prevalence was found in gut isolates of households (66.67%) and lowest (40.00%) in both external and gut isolates of household and restaurants. Among the category of samples the variation in prevalence is not statistically significant (p>0.05).

Table-4.2: Prevalence of antimicrobial resistance pattern of Staphylococcus isolated from external washings

Antibiotics	Staphylococcus positive isolates	Pattern		
		Resistance (%)	Intermediate (%)	Sensitive (%)
Erythromycin	11	45.45	27.27	27.27
Kanamycin	11	0	0	100
Penicillin	11	72.72	0	27.27
Oxacillin	11	45.45	9.09	45.45
Cephalothin	11	0	0	100
Clindamycin	11	27.27	72.72	0

The results of antimicrobial resistance pattern against Staphylococcus isolated from external washings are given in Table-4.2. The results revealed that the isolates from external washings were highest resistance to Peniicillin (72.72%) followed by Erythromycin (45.45%), Oxacillin (45.45%) and others (0-50%). Kanamycin and Cephalothin showed highest level of sensitivity (100%) followed by Oxacillin (45.45%) and Penicillin (27.27%). The increasing rates of resistance to Erythromycin and Clindamycin among the isolates might attributed to the emergence of multi resistance Staphylococcus spp. Figure-4.1 shows pattern of Staphylococcus isolates from external washings.

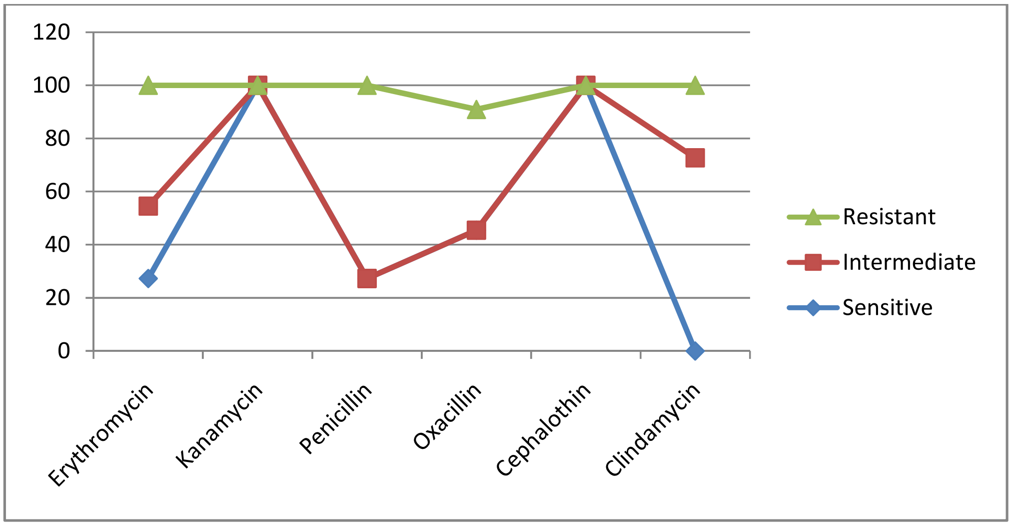


Figure-4.1: Resistance pattern of *Staphylococcus* isolated from external washings.

Table-4.3: Prevalence of antimicrobial resistance pattern of Staphylococcus isolated from Gut

Antibiotics	Staphylococcus positive isolates	Pattern		
		Resistance (%)	Intermediate (%)	Sensitive (%)
Erythromycin	14	64.28	14.28	21.42
Kanamycin	14	0	0	100
Penicillin	14	85.71	0	14.28
Oxacillin	14	21.42	21.42	57.14
Cephalothin	14	0	0	100
Clindamycin	14	78.57	14.28	7.14

In this study, all the isolates were tested for the susceptibility against 6 different commercially available antimicrobial discs which showed in the table-4.3. Resistance spectrum of *Staphyloccus* for 6 antibiotics tested were Erythromycin (64.28%), Penicillin

(85.71%), Oxacillin (21.42%), and Clindamycin (78.57%), whereas Kanamycin and Cephalothin is not resistant. On the other hand, Kanamycin and Cephalothin showed highest (100%) sensitivity followed by Oxacillin (57.14%) and othes (0-50%). Resistance pattern of *Staphylococcus* isolated from gut content were given in figure- 4.2.

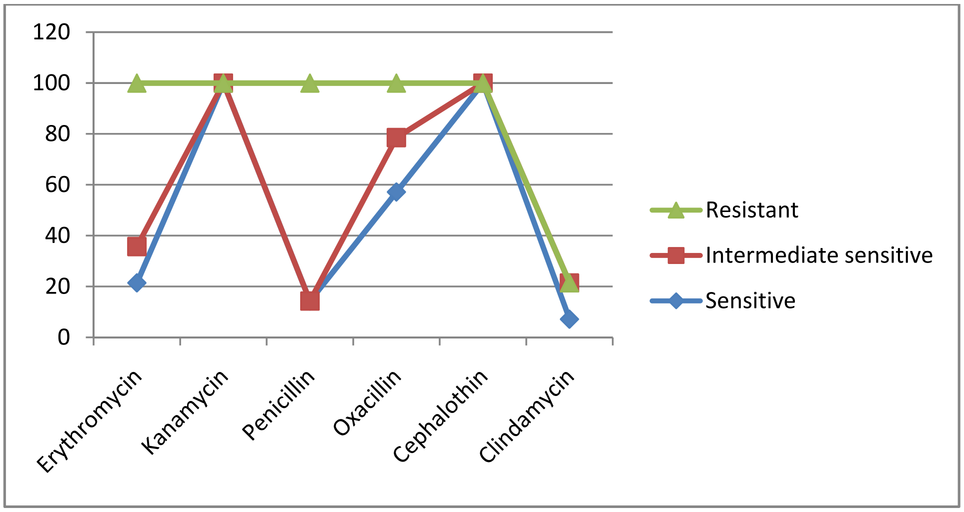


Figure-4.2: Resistance pattern of *Staphylococcus* isolated from Gut.

Table- 4.4: Patterns of multidrug resistance in isolates of *Staphylococcus* in different sample

Antimicrobials	Pattern	External n=11	Gut n=14	Range	Average
Erythromycin	R	45.45	64.28	45.45-64.28	54.86
	I	27.27	14.28	14.28-27.27	20.78
	S	27.27	21.42	21.42-27.27	23.35
Kanamycin	R	0	0	0	0
	I	0	0	0	0
	S	100	100	100	100
Penicillin	R	72.72	85.71	72.72-85.71	79.22
	I	0	0	0	0
	S	27.27	14.28	14.28-27.27	20.78
Oxacillin	R	45.45	21.42	21.42-45.45	33.44
	I	9.09	21.42	9.09-21.42	15.25
	S	45.45	57.14	45.45-57.14	51.30
Cephalothin	R	0	0	0	0
	I	0	0	0	0
	S	100	100	100	100
Clindamycin	R	27.27	78.57	27.27-78.57	52.92
	I	72.72	14.28	14.28-72.72	43.50
	S	0	7.14	0-7.14	3.57

Among the six tested antimicrobials resistance against *staphylococcus* isolates Penicillin turned out as the highest level of resistance ranged at (72.72-85.71%) followed by Erythromycin (45.45-64.28%), Clindamycin (27.27-78.57%), and Oxacillin (21.42-45.45%). The rate of sensitivity to individual antibiotics against *Staphylococcus* isolates from different sampling sites was highest in Kanamycin and Cephalothin (100%) followed by Oxacillin (45.45-57.14%), Erythromycin (21.42-27.27%), Penicillin (14.28-27.27%) and lindamycin (0-7.14%). Patterns of multidrug resistance isolates of *Staphylococcus* in different isolates presented graphically in figure- 4.3.

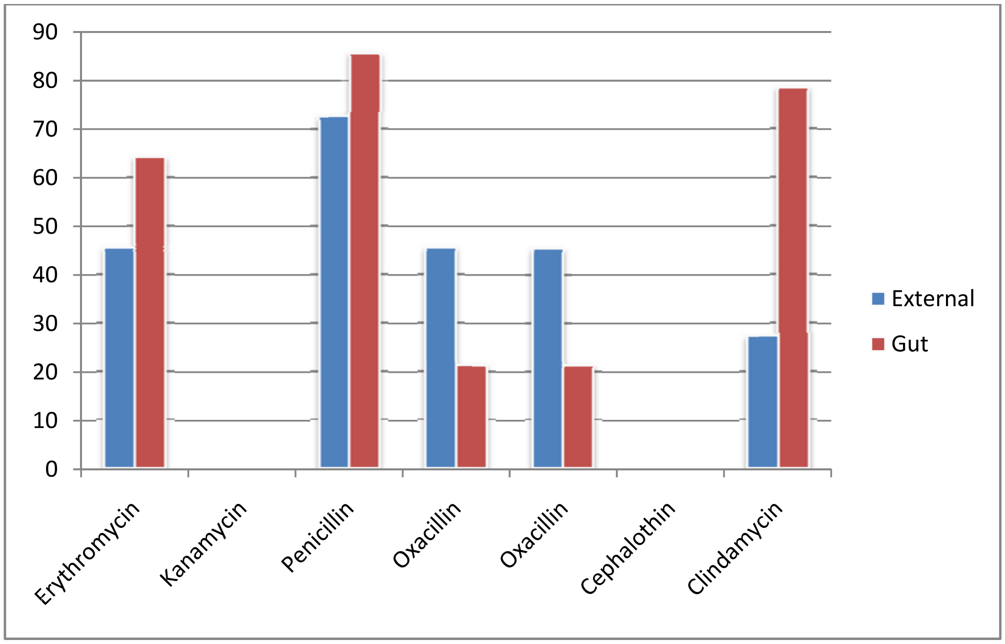


Figure- 4.3: Patterns of multidrug resistance *Staphylococcus* isolates in different source

Chapter-5: Discussion

Cockroaches are among the most notorious pests of premises, which do not only contaminate food by leaving droppings and cause food poisoning but also transmit bacteria and other pathogenic microorganisms in infested areas (Kopanic, 1994; Czajka *et al*., 2003). Cockroaches feed indiscriminately on garbage and sewage and so have copious opportunity to disseminate human pathogens (Cotton *et al.,* 2000, Pai *et al*., 2005).

5.1 Prevalence of Staphylococcus from gut contents and external washings of cockroach

The results of this study demonstrated a high prevalence of *Staphylococcus* among the samples investigated in the external washings and gut contents. These results are similar to the study which is done on clinical and non-clinical samples of Bovine and Ovine animals and revealed more than 50% positive for *Staphylococcus* (Bamaiyi *et al.,* 2013). These results are similar to those reported from Umudike, Nigeria (Achi and Ugbogu, 2006) and also found prevalence more than 50%. The result is also an indication that *Staphylococcus* is one of the most common pathogen in most infections which is in agreement with the report of earlier workers (Werckenthin *et al.,* 2001; Oranusi *et al.,* 2003; Olaoye and Onilude, 2009; de la Fuente *et al.,* 2011). Our isolation of *Staphylococcus* from *P. americana* collected from restaurants and households is in agreement with other findings elsewhere (Paul *et al.,* 1992; Oothuman *et al.,* 1989; Burgess 1984; Le Guyader *et al.,* 1989; Prado *et al.,* 2002). The isolation of almost proportional numbers of *Staphylococcus* from household and restaurant cockroaches is indicative of the potential role of cockroaches in the dissemination of *Staphylococcus* in Restaurants and household foods alike.

5.2 Antimicrobial resistance

The use of antimicrobials in food animals has resulted in the development of antimicrobial resistance (White *et al.,* 2001), through mutation and acquisition of resistance encoding genes (Fluit, 2005). The situation in developing countries like Bangladesh may be exaggerated by easy accessibility of antimicrobials at a cheaper price and their extensive use in poultry production system (Prakash *et al.,* 2005). Another major setback might be the quality and potency of locally produced antimicrobial drugs; for example, there are over 80 different brands of the Fluoroquinolones (Ciprofloxacin) in Bangladesh. Thus there is widespread availability and uncontrolled use of antibiotics poses the antimicrobial resistance in food animals and their products which is the actual threat of public health.

5.3 Level of antimicrobial resistance *Staphylococcus* from gut contents and external washings

5.3.1 Erythromycin

In this study Erythromycin was resistance higher against *Staphylococcus* spp. isolated from gut contents and external washings even that are higher than the resistance of Erythromycin (39.1%) reported in Uyo, Akwa Ibom State (Akinjogunla, OJ *et al.,* 2012). Another study shows the resistance of Erythromycin against *Staphylococcus* spp. was 28.1% reported in Maiduguri, Nigeria (Bamaiyi *et al.,* 2013). It could be happened due to heavily use of Erythromycin against different infectious diseases including Staphylococcosis without proper diagnosis of diseases or proper maintaining of drug doges. Resistance of Erythromycin was evidenced as a serious problem in the study sites and it might be due to subnormal doses and incomplete treatment course of Erythromycin.

5.3.2 Penicillin

In this study, Penicillin was resistance against *Staphylococcus* spp. isolated from gut contents and external washings which is similar to the resistance of Penicillin (81.7%) reported in Maiduguri, Nigeria (Bamaiyi *et al.,* 2013). Another study shows the resistance of Penicillin against *Staphylococcus* spp. was 27.3% reported in Uyo, Akwa Ibom State (OJ. Akinjogunla *et al.,* 2012).So our present study shows higher resistance of Penicillin than the study done in Uyo. It may be due to common use of Penicillin in skin diseases of livestock and Human. The high resistance of Penicillin to *Staphylococcus* spp. from external and gut contents of cockroach of present study might be due to indiscriminate use of these antibiotics to human as well as in livestock. In addition, sometimes people select drugs by their own or rely on neighbors and these mal-practices may not always ensure proper drugs doses, frequency of drug administration and complete course of drug treatment. The above factors might have influenced on Penicillin to be resistance against *Staphylococcus* spp.

5.3.3 Clindamycin

Resistance to Clindamycin against *Staphylococcus* was found comparatively higher in the present study as compared to (41.50%) resistance in Maiduguri, Nigeria (Bamaiyi *et al.,* 2013) . it is due to indiscriminate use of this antibiotic.

5.3.4 Oxacillin

Resistance of Oxacillin in this study was lower. In the study of Maiduguri, Nigeria which was done on clinical and non-clinical samples of Bovine and Ovine animals shows Oxacillin is completely sensitive to the *Staphylococcus* spp (Bamaiyi *et al.,* 2013). Oxacillin is also commonly use in livestock, poultry and Human treatment regularly. The high resistance of Oxacillin to *Staphylococcus* spp. from external and gut contents of cockroach of present study might be due to indiscriminate use of these antibiotics to human as well as in livestock.

5.3.5 Kanamycin

Kanamycin showed full sensitivity in present study which is good news for the People of Bangladesh because it can say from the result kanamycin is usable until now in different treatment.. Kanamycin was less widely used in treatment of livestock and human in Bangladesh that's why kanamycin resistant *Staphylococcus* spp. was not available.

5.3.6 Cephalothin

Cephalothin showed full sensitivity in present study, But in a similar study of Uyo, Akwa Ibom State shows that Cephalothin has lower resistance (24.20%) against *Staphylococcus* spp (Akinjogunla, OJ *et al.,* 2012). It is good news for the People of Bangladesh because it can say from the result Cephalothin is usable until now in different treatment. Cephalothin was less widely used in treatment of livestock and human in Bangladesh that's why Cephalothin resistant *Staphylococcus* spp. was not available.

5.4 Resistance pattern

Overall, four antimicrobial resistance were observed among *Staphylococcus* isolated from gut contents and external washings *Staphylococcus* spp. was resistant to four of the six antimicrobials tested with simultaneous multi-drug resistance to antimicrobials. A similar study in Uyo, Akwa Ibom State showed higher multi-drug resistance in *Staphylococcus* spp isolates from cockroach (Akinjogunla, *et al.,* 2012) *Staphylococcus* spp was also found to be comparatively resistant to as many as ten drugs tested in Maiduguri, Nigeria (Bamaiyi *et al.,* 2013). The increasing rates of resistance to Penicillin, Erythromycin, Clindamycin and Oxacillin among the isolates might be attributed to the emergence of multi resistance *Staphylococcus* spp.

Chapter-6: Conclusion

Staphylococcosis is one of the leading food-borne diseases worldwide. A wide range of foods has been implicated in such disease. However, foods contaminated by staphylococcus which is frequently carried by cockroach implicated in sporadic cases and outbreaks of human Staphylococcosis. The results of the present study indicate that *Staphylococcus* contaminated foods are common in household and restaurant of Chittagong, Bangladesh. The poor storage and handling of foods at home and the site of sale of restaurant could be a source of contamination. In the current study the prevalence of *Staphylococcus* spp. was higher in hosehold due to aboundent presence of cockroach. *Staphylococcus* and antibiotic resistance was a big problem in Bangladesh. In the current study, *Staphylococcus* isolates displayed resistance to antimicrobial and displayed a larger number of multiple resistances. From the findings of the study, it is avowed that a larger number of resistant isolates of *Staphylococcus* for Erythromycin, Penicillin, Oxacillin, and Clindamycin. The excess utilization of antibiotics in *staphylococcal* disease might be the cause of increased resistance. Kanamycin and Cephalothin identified as sensitive drugs and should be used to treat Staphylococcosis carefully. Cares should be taken to maintain the sensitivity of the drugs selecting the correct drugs, proper doses and complete course of treatment. This multiple antimicrobial-resistant nature of the organism adds to the gravity of the problem. The level of resistance of *Staphylococcus* to antibiotics should be alarming to the food storage, processing, distribution and handling of food product .Therefore, it is necessary to inform people involved in the processing of food to take care in storage and handling of the food products. It is also necessary to aware them about the importance of cleanliness and in the the total procedure from storage to delivery of food products and harmful effects of cockroach associated with food. The present situation underlines the increased public and governmental interest in eliminating sub-therapeutic use of antibiotics used to treat humans and animals. There is need for more rational use of antibiotics in animal production and more prudent use in humans. It is important to take concerted action to improve antibiotic resistance surveillance capacity worldwide with a view to monitoring the emerging resistance genes and their transfer in both animal and human. In addition, alternatives to antibiotics should be explored such as the application of probiotics.

Chapter-7: Recommendation

The current study reveals presence of *Staphylococcus* in the cockroach collected from different household and restaurants of Chittagong City Corporation of Bangladesh. Isolation of antimicrobial resistant *Staphylococcus* spp. including multi-drug resistance poses a concern to public health authorities and the general Bangladeshi people. In view of this research finding, there is a need to develop comprehensive policies to ensure safe food and prevent multi-drug resistant. The following recommendations should prove useful to prevent the contamination of food and its environment and to ensure the judicious uses of antibiotics.

7.1 Recommendations for food producers

These points for good management practice must be strictly adhered to by food producers:

1. Applying proper storage capacity of food to avoid cockroach as well as to prevent environmental contamination

2. Take proper step to control the pests like cockroach, flies etc. and ensure that the feed storage room is free of insets especially from cockroach

3. Premises and equipment for handling and storage of food must be maintained in a sanitized state fit for the production of food for human consumption

4. It is important to wash the raw food properly with clean water as water contains many harmful organisms

5. Dirty or contaminated foods must be separated from clean food as soon as possible to minimize contamination

6. It is illegal to sell contaminated and stale food to the consumers. It is essential to check the foods before sell. Stale foods must be disposed

7.2 Recommendations for consumers

To minimize the potential risk of Staphylococcosis due to the consumption of foods and to be safe they must be safely stored, handled and cooked:

1. Avoid eating unwashed raw ingredients as they are more likely to be contaminated and thus present a higher health risk

2. Always try to take the food which is fresh and devoid of any type of dirt

3. Feeds with dirt should be washed. Washed feed should then be used as soon as possible

4. Eating raw and feed with bad odor should be avoided

5. Hands, cooking utensils, and food-preparation surfaces should be washed with hot water and soap after contact with contaminated foods

7.3 Recommendations for practitioner

To prevent the multidrug resistant *Staphylococcus* spp. Humans and practicing veterinarians should follow the following recommendations:

1. Infection by such multi-drug resistant *Staphylococcus* may no longer be treated by conventional therapeutic agents

2. Sensitive drugs should be identified and choice to treat Staphylococcosis

3. Care should be taken to use the sensitive drugs judiciously in terms of dose, frequency of administration and course of drug treatment

7.4 Research recommendations

1. In order to control *Staphylococcus* infection of in Bangladesh detailed epidemiological investigation and strain identification are prerequisites

2. As the results from this single investigation are not sufficient for formulating standards by the regulatory agencies, more large-scale studies are required to explore prevalence of *Staphylococcus* in cockroach and their environment and antibiogram against *Staphylococcus* spp. isolates

3. Further studies on the national level to identify *Staphylococcus* serotypes and specific antimicrobial resistant gene of *Staphylococcus* isolates should be needed

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