

Original Article

Isolation, identification and antimicrobial resistance profile of *Staphylococcus aureus* in Cockroaches (*Periplaneta americana*)

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ABSTRACT

Objective: The study was conducted to determine the prevalence of *Staphylococcus aureus* in cockroaches (*Periplaneta americana*), and to assess the antimicrobial resistance profiles of the isolated bacteria.

Materials and methods: A total of 150 cockroaches (*P. americana*) were randomly captured from three households and four restaurants in Chittagong City Corporation, Bangladesh during July to December 2014. The cockroaches were transported to the bacteriology laboratory at the Poultry Research and Training Centre (PRTC), Chittagong Veterinary and Animal Sciences University. The isolation and identification of *Staphylococcus spp.* from the external surface wash and gut homogenates by pooling cockroaches were done by following conventional bacteriological examinations followed by biochemical characterization. The antibiotic susceptibility profiles of the isolates were determined using disc diffusion method.

Results: In this study, the overall prevalence of *S. aureus* was 38% (n=57/150). Higher prevalence of *Staphylococcus spp.* was observed among the cockroaches from restaurant (49.3%; n=37/75) as compared to those of households (26.7%; n=20/75) having a significant difference ($P<0.05$). Highest level of resistance by the *Staphylococcus spp.* was found to Penicillin (68%) followed by Erythromycin (60%), Oxacillin (46%) and Clindamycin (31%). On the other hand, the *Staphylococci* isolates were highly sensitive to Cephalothin (84%) and Kanamycin (65%).

Conclusion: The rational use of antibiotics needs to be adopted in both human and animal medicine practices to prevent the emergence of drug resistant *Staphylococcus spp.*

KEYWORDS

Antibiogram, Cockroach, External washing, Gut content, Prevalence, Resistance

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INTRODUCTION

Cockroach is one of the most common pests in urban environmental settings that are associated intimately to the food which carries and spread antimicrobial resistant bacterium frequently ([Bennett and Owens, 1986](#)). Cockroaches are largely found in multi-family dwellings, and may act as a carrier of several microorganism affecting public health ([Wood et al., 1981](#)). Both in restaurants and households, cockroaches are commonly found in dark rooms, kitchens, bathrooms and food storage rooms, and they have the ability to move from one part of a building to another and even in dark light ([Rivault, 1989](#)). The cockroaches come in contact with garbage, feces, stored food, sewage, and biological wastes ([Rivault et al., 1993](#)). Close association to the human dwellings, contact with environmental wastages might have influence in the emergence and spread of antimicrobial resistant *Staphylococcus aureus* from cockroaches to human and vice versa.

Emergence and rapid spread of antimicrobial resistance bugs particularly *S. aureus* has become a grave global concern. Multidrug resistant (MDR) *Staphylococcus* poses a growing problem for human health and has been considered as horrifying public health threat ([Ahaduzzaman et al., 2014](#)). The dismaying bug is commonly found within tissues, skin surfaces or in foods contaminated by exposure to infected human beings or animals. Close interface between human, animal, and environmental components both biological and physical, the threat of pathogenic Staphylococci is continuously surging up towards a complex situation ([Tachbele et al., 2006](#)). *Staphylococcus spp.* is recognized as a foremost cause of food poisoning, wound infections, skin infections, infections of internal organ, and once resistant *S. aureus* emerges in such infections, it will be very difficult to continue treatment regimens, because bacterium may acquire resistance against commonly used drugs. Foods are usually contaminated by the different pathogens due to unhygienic handling of food results. Close contact of human and animal is one of the possible sources of food contamination, and spreading of pathogen to foods or utensils through rodents and pests such as cockroaches.

Around the globe, many researchers reported that while living close to human dwellings cockroaches play vital roles in transmitting pathogens like bacteria, virus, helminthes and protozoan ([Agbodaze and Owusu, 1989](#); [Fotedar et al., 1991](#); [Cloarec et al., 1992](#); [Pai et al., 2003](#)), and some of isolated bacterial pathogens were resistant to various antimicrobials ([Fathpour et al., 2003](#)). Different types of food sources contain resistant bacteria that are resistant to one or more antimicrobials that are used in

human or veterinary medicine, and for the production of food-animal ([Bager and Helmuth, 2001](#); [Anderson et al., 2003](#); [Schroeder et al., 2004](#)). These resistant microbes may act as a potential source in spreading antimicrobial resistance gene to human pathogens ([Schroeder et al., 2004](#)).

The isolation and identification of cockroach associated with *Staphylococcus spp.* and their antimicrobial resistance profile are of public health importance and imperative to the scientific community to take appropriate strategies and could provide information on the supplementary emergence of drug resistance. Therefore, the present research was aimed to assess the prevalence and antibiogram profile of *S. aureus* isolated from cockroaches in restaurants and households.

MATERIALS AND METHODS

Cockroach collection and identification: Three household and four restaurants of Chittagong City Corporation (CCC) were randomly selected where large number of people had their foods every day. The households had at least six members and the restaurants represented medium level eating centers which attended around three hundred and fifty customers per day. Sampled cockroaches were collected using sterile screw-capped 250-mL jars and sterile hand-gloves ([Paul et al., 1992](#)), and were transported to the “Poultry Research and Training Centre” (PRTC), Chittagong Veterinary and Animal Sciences University (CVASU) for bacteriological analyses. The insect identification has been confirmed in accordance with [Lane and Crosskey \(1993\)](#).

Estimation of sample size: The required sample size was based on the prevalence of 13% (n=24/187) *S. aureus* among isolated bacterial pathogens in conserved cockroaches of hospitals and dwelling of Northeast Algeria ([Tine et al., 2014](#)). However, we are assuming that cockroaches might show 10% Staphylococcal prevalence in the study population. Based on this assumption, we estimated that 150 cockroaches would be sampled for our study (population size 100000, confidence limits 5%, design effect 1.0 and clusters 1). The sample size was calculated using windows version of the Epi Info™ 7.1.5.0 software.

Processing of cockroaches to isolate *Staphylococcus aureus*: Captured cockroaches humanely killed using chloroform soaked cotton in a screw capped sterile jar. Body surface of cockroaches were washed with physiological saline after vortexing for 2 min and taken as a homogenate sample. Before gastrointestinal tract (GIT) dissection, each cockroach was decontaminated with 95%

Table 1. Antibiotics used, concentrations and zone diameter interpretative standards based on [CLSI \(2007\)](#)

Antimicrobial	Antibiotics	Concentration	Zone diameter (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

R=Resistant, I=intermediately resistant, S=Sensitive

ethanol, and the residue of ethanol was removed with saline solution. The gut was dissected aseptically using sterile needles and washed with 5-mL normal saline solution. Caution was taken to reduce the number of cut off or break in the gut. 1-mL of each homogenate was inoculated separately into 9-mL of buffered peptone water (BPW) (OXOID, Basingstoke, UK) for primary enrichment, and incubated at 37°C for 18-24 h.

Cultural identification: The samples were inoculated in screw cap test tube containing nutrient broth (OXOID, Basingstoke, UK) for the isolation of *S. aureus*, and enriched overnight at 37°C. A loopful of inoculum from the enriched culture was streaked on to Blood Agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 h for the observation of hemolysis. Growth of yellow colonies on Mannitol Salt Agar (MSA; Oxoid Ltd, Basingstoke, Hampshire, UK) surrounded by yellow zones as a result of fermentation of mannitol after 24 h of incubation at 37°C indicated a positive result ([Kateete et al., 2010](#)). Smear was prepared on clean grease free microscopic glass slide from the isolated colony and Gram staining was done ([Islam et al., 2007](#); [Gulani et al., 2016](#); [Abdelkhalek et al., 2016](#)), and was observed under microscope ([Thaker et al., 2013](#)). All the positive samples were subjected to coagulase and catalase tests for biochemical confirmation of *S. aureus*, as described by [Cheesbrough \(2006\)](#).

Catalase test: A sterile loop was used to collect pure culture of each isolate from the agar slant and was mixed with a drop of 3% H₂O₂ on a clean glass slide, and bubbles of oxygen liberated within a few seconds were indicated the positive result ([Cheesbrough, 2006](#)).

Coagulase tests: Slide and tube coagulase tests were performed as per the procedure described by [Cheesbrough \(2006\)](#).

Antibiotic sensitivity analysis: After the confirmatory identification by cultural and biochemical tests, all positive Staphylococcal isolates were investigated for antimicrobial susceptibility. Bauer-Kirby disk-diffusion

procedure was used on Mueller-Hinton (MH) agar ([Bauer et al., 1966](#)), and a 0.5 McFarland standard was prepared by adding 0.5 mL of 1% (11.75 g/L) BaCl₂.2H₂O to 99.5 mL of 1% (0.36 N) H₂SO₄ ([Carter and Cole Jr., 2012](#)). The **Table 1** shows the panel of antibiotics used for the assays and the size of zone of inhibition of them to be considered as “resistant (R)”, “Intermediately resistant (I)” and “sensitive (S)”, and the interpretations were made based on the recommendations from Clinical and Laboratory Standards Institute ([CLSI, 2007](#)).

Ethical approval: In Bangladesh, cockroaches are considered as vermin and the current research protocol was approved by Animal Ethical Experimentation Committee (CVASU-AEEC) of CVASU, Bangladesh.

Data analysis: Field and Laboratory data were stored and cleaned in the Excel 2007 Microsoft office program before exporting to STATA/IC-13.0 for analysis. Descriptive analysis was performed to discern the frequency and distribution of *Staphylococcus* and antibiotic resistance patterns.

RESULTS

A total of one hundred and fifty samples were processed from both households (n=75) and restaurants (n=75). Of these samples, 57 isolates were confirmed to be associated with *S. aureus* based on cultural and biochemical analyses, as stated in **Table 2**.

The prevalence of *S. aureus* was 26.67% (n=20/75) and 49.33% (n=37/75) in the samples collected from household and restaurant respectively with an overall prevalence of 38% (n=57/150) as shown in **Table 3**. Statistically significant difference ($P<0.05$) was observed between sampling sites (household and restaurant). Considering the two different sample collection sites (*i.e.*, external washing and gut contents) in the same cockroach sample, higher prevalence of *S. aureus* was documented in gut content (26.67%; n=20/75) of the cockroaches originated from restaurants as compared to that of external washing (22.67%; n=17/75) ($P<0.05$). (**Table 4**).

Table 2. Cultural and biochemical profile of Staphylococcal isolates

Culture media	Phenotypic characteristics	Biochemical tests	Phenotypic characteristics
Blood agar	On blood agar, colonies of <i>S. aureus</i> were circular, small, smooth raised with gray white or yellowish in color, and β -hemolysis was evidenced.	Tube coagulase test	Heavy coagulation of all the contents of the tube and did not come off even after inverting the tube upside down
Mannitol Salt agar (MSA)	<i>S. aureus</i> fermented MSA with the production of yellowish colonies	Slide coagulase test	Agglutination of culture in slide test
		Catalase test	Gas bubble due to oxygen production has been noticed

Table 3. Prevalence of *Staphylococcus spp.* in the sampled cockroaches

Variables	Sample (n)	Positive (%)	P-value
Household	75	20 (26.66)	0.0043
Restaurant	75	37 (49.33)	
Total	150	57 (43.00)	

A P value of <0.05 was considered as significant

Table 4. Prevalence of *Staphylococcus spp.* in different anatomical sampling sites

Sample collection site(s)	Categories of location	Positive (%)	P-value
External washing	Household	07 (09.30)	0.0264
	Restaurant	17 (22.67)	
Gut contents	Household	13 (17.33)	0.1690
	Restaurant	20 (26.67)	

A P value of <0.05 was considered as significant

In this study, all the isolates were tested for the susceptibility against 6 different commercially available antimicrobial discs (**Table 5**). Resistance patterns of *S. aureus* against the antibiotics tested were- Erythromycin (60 and 58%), Penicillin (68 and 71%), Oxacillin (45.50 and 45.50%), and Clindamycin (30.50 and 38%), in both household and restaurant, respectively. On the other hand, Kanamycin (65 and 58%) and Cephalothin (84 and 66%) were found to be sensitive shown by the *S. aureus* in both sampling sites.

DISCUSSION

Cockroaches identified as potential mechanical vector for bacterial pathogen dissemination in health-care facilities (Uçkay et al., 2009; Zarchi and Vatani, 2009; Oliva et al., 2010; Pai, 2013), restaurant and dwelling environments (Tachbele et al., 2006; Sayyad et al., 2016). However, no such information was yet available on household and restaurant cockroaches in Bangladesh. Of the 7 households and restaurants were surveyed in the study, 100% were evident to have cockroach (*P. americana*) infestation. This infestation rate was found higher than those reported in dwelling environment in Taiwan (50%)

and Iran (54.1%) (Pai et al., 2005; Sayyad et al., 2016). Identified cockroaches were commonly infested in toilets, kitchen, food cabinet, ceilings, food processing and garbage units of the household and restaurants. Though there was difficulty to validate this study findings (such as species identification, roaming territory and mechanical vector potentiality of cockroach) in the absence of earlier work on cockroaches in Bangladesh, the findings of this study was sufficient to indicate that cockroach invasion was neglected in restaurant and household environment in Bangladesh.

In this study, about 38% of household and restaurant Americana cockroaches has been isolated with drug resistant gram positive bacteria *S. aureus* (57 out of 150). However, higher *S. aureus* isolation rate (72%) was documented in household cockroach of Ahvaz province, Iran (Kassiri et al., 2014). Besides this, *S. aureus* was also reported from hospital Americana cockroaches (24.6% to 60%) in Iran (Feizhaddad et al., 2012; Hamid and Shahnaz, 2012). By comparison, we can assume that cockroaches present in hospital environment in Bangladesh may also likely to be found with *S. aureus*. Isolation of *S. aureus* in this study from household and restaurant cockroaches supports the findings of earlier studies (Burgess, 1984; Le Guyader et al., 1989; Oothuman et al., 1989; Paul et al., 1992; Prado et al., 2002). Previously, multiple bacterial species of public health significance were isolated from Americana cockroach by other authors; the isolated bacteria were- *Enterobacter* (19.2-53.3%), *Streptococcus* (15.1-60%), *Klebsiella* (47.9%), *Proteus* (2.7-73.3%), *Pseudomonas* (37%), *Escherichia coli* (30.1-86.7%), *Shigella* (33.3%), *Staphylococcus* (6.7-60%), *Serratia* (8.2-13.3%) and *Bacillus* (4.1-66.7%) (Feizhaddad et al., 2012; Hamid and Shahnaz, 2012). Though our study did not confirm other bacterial species, due to time and budget constraint, confirmation of *S. aureus* both in household and restaurant cockroaches is suggestive of the prospective role of cockroaches in the dissemination and contamination of environment with those public health significant bacteria through Americana cockroach's species in Bangladesh.

Table 5. Numbers of isolates showing resistance, intermediate resistance and sensitive to antimicrobials used (households and restaurants)

Antimicrobials	Household (N=20)			Restaurants (N=37)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Erythromycin	12 (60.00)	03 (15.00)	05 (25.00)	21 (58.00)	10 (27.00)	06 (15.00)
Kanamycin	04 (21.00)	03 (14.00)	13 (65.00)	09 (23.00)	07 (19.00)	21 (58.00)
Penicillin	14 (68.00)	02 (09.50)	04 (22.50)	26 (71.00)	07 (19.00)	04 (10.00)
Oxacillin	09 (45.50)	03 (15.00)	08 (39.50)	17 (45.50)	09 (25.00)	11 (29.50)
Cephalothin	01 (05.00)	02 (11.00)	17 (84.00)	04 (11.00)	09 (23.00)	24 (66.00)
Clindamycin	06 (30.50)	13 (67.00)	01 (02.50)	14 (38.00)	21 (57.00)	02 (05.00)

R=Resistant, I=Intermediately resistant, S=Sensitive

A prevalence of 35.16% of *S. aureus* was reported in hospital settings in Brazil (Oliveira et al., 2014). Similarly, 44% prevalence was reported in hospital settings from Iran (Heidari et al., 2015). In Taiwan, 46.7% clinical and nonclinical samples were found to be contaminated with *S. aureus* (Pai et al., 2004).

The indiscriminate use of antimicrobials in food producing animals has resulted in the development of antimicrobial resistance bacteria through mutation and attainment of resistance encoding genes (White et al., 2001; Fluit, 2005). The circumstances in developing countries like Bangladesh may be inflated by easy convenience of antimicrobials at a cheaper price and their extensive use in animals (Prakash et al., 2005) as well as in all livestock production system. Another major setback might be the quality and effectiveness of locally produced antimicrobial drugs; for example, there are 80 or more different brands of the Fluoroquinolones (Ciprofloxacin) in Bangladesh. Thus, the widespread availability and indiscriminate use of antibiotics made a risk of developing antimicrobial resistance in food animals and their products.

The MDR *Staphylococcus* in cockroach was reported earlier from Nigeria, Ethiopia, Iran, and Brazil (Tachbele et al., 2006; Salehzadeh et al., 2007; Akinjogunla et al., 2012; Pai, 2013; Menasria et al., 2014). In this study, we have confirmed only one gram positive MDR *S. aureus*. Our isolation of *S. aureus* from household and restaurants, cockroach has showed resistance against Penicillin, Erythromycin, Oxacillin and Clindamycin and shown a common agreement with earlier studies (Oothuman et al., 1989; Paul et al., 1992; Prado et al., 2002). In this study, Kanamycin and Cephalothin were found to be sensitive against *S. aureus* isolates. However, Tachbele et al. (2006) reported that *S. aureus* was resistant of Kanamycin and Cephalothin in Ethiopia. In addition, resistance was also found against other antibiotics such as Ampicillin, Sulfamethoxazole, Polymyxin B, Carbenicillin, Chloramphenicol, Streptomycin, Tetracycline, Augmentin, Clindamycin, Oxacillin, Erythromycin, Penicillin-G, Vancomycin,

Mupirocin in Ethiopia (Tachbele et al., 2006). Studies also documented isolation of other MDR bacterial pathogens such as *Salmonella spp.*, *Shigella spp.*, *E. coli* 0157, *Bacillus cereus*, *Pseudomonas aeruginosa* and *S. aureus* (Tachbele et al., 2006; Menasria et al., 2014).

Though this study tested MDR against single bacterium, in the absence of prior work, the findings of this study would signify the emergence of MDR *S. aureus* in the environment and the prospective likelihood of dissemination of such strains through mechanical vector Americana cockroaches in Bangladesh.

In this study, Kanamycin and Cephalothin were sensitive against *S. aureus*. However, these two antibiotics were reported as completely resistant in Ethiopia (Tachbele et al., 2006). This variation might be due to difference in public awareness and government regulation of drug use.

Staphylococcus spp. isolated from cockroaches of household and restaurant were resistant to 4 out of 6 antimicrobials, indicating that the MDR *S. aureus* are prevalent among cockroaches. The MDR *Sytayphylococcus* in cockroach also reported from others countries like Nigeria, Ethiopia, Iran and Brazil (Tachbele et al., 2006; Akinjogunla et al., 2012; Menasria et al., 2014).

CONCLUSION

The results of the present study indicate that *Staphylococcus* contaminated foods are common in households and restaurants of Chittagong, Bangladesh. *Staphylococcus* and its growing antibiotic resistance become a crucial problem in Bangladesh. Cares should be taken in selecting the appropriate antibiotics, and doses and complete course of treatment should also be maintained. The level of resistance of *Staphylococcus spp.* to antibiotics indicates a considerable threat for human and animal. Therefore, necessary cares are essential during food processing, handling by the workers and storage so that cockroaches can not spread the bacteria.

CONFLICT OF INTEREST

Nothing to declare.

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AUTHOR CONTRIBUTIONS

The research ideas were generated by AI and MMH. They were also responsible for supervision of all research activities. ADN and AAF did the laboratory analyses. The statistical analysis and paper drafting were carried out by AI, MMH, KI, SC, SI and MBH.

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