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GRAM-NEGATIVE BACTERIA LOAD AND MULTIPLE ANTIBIOTIC RESISTANCE (MAR) PATTERN OF PATHOGENIC BACTERIA ISOLATED FROM COCKROACHES AT HABOUBI HOSPITAL IN THE PROVINCE OF DHI –QAR

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ABSTRACT

This study investigates the bacterial load and antibiotic susceptibility pattern of pathogenic bacteria isolated from the guts and body surfaces of cockroaches in Haboubi Hospital, the province of Dhi Qar. *Periplaneta americana* collected from hospital were screened for microbial load and antibiotic susceptibility pattern using standard protocols. The identified bacteria isolates and their percentage distribution, It is 152 bacterial isolates the external surfaces of 98 (65%) and guts of 54 (35 %) belonged to the group of Gram-negative bacilli, The distribution of the isolated bacteria includes the highest value to *Salmonella .Spp* (20) and *Proteus mirabilis* (19),While the lowest value of *Aeromonas .hydrophila /coviae / sobria1* and *Raoultella.ornithinolyticae* (1). The microbial load were higher in the outer surface (94%) as compared with guts (6%). ($p<0.05$) Percentage Gram Negative bacterial loads isolated from Cockroaches reached the highest loads to the *Serraciae marcescens* (11.6%) Followed by *Pro.mirabilis* (11.2%) while less loads *A.hydrophila/coviae/Sobria* (0.7%) and *Rao.ornithinolyticae* (1.1%) . The isolates demonstrated high level resistance to PY (79 %), AMC (73.6%) and CRO(73%) while VA(93.4%) was the most effective of all the antibiotics used. The bacteria isolates have high percentage multiple antibiotic resistance (MAR) ranging from(33.3 % - 100%), *Citrobacter brakii* have the least value of 33.3 %, while the some isolates demonstrated 100 % multiple antibiotic resistance patterns, efforts geared towards controlling the cockroaches will be indispensable in curbing the wide spread

of multi-drug resistant pathogens in the study area. These findings suggest cockroaches in Haboubi Hospital as potential vectors of medically important multiple drug-resistant bacteria.

KEYWORDS: bacterial load, multi-drug resistance, hospital, cockroaches.

INTRODUCTION

Cockroaches are among the varieties of medically important insects found in urban and rural environments that cause serious public health problems[12]. They have been found to harbour diverse pathogenic bacteria, different protozoan, pathogenic worms, fungi, and viruses on either the cuticle or in the gut[14]. Cockroaches can carry up to 14 million bacteria on the body and 7 million in each of their faecal droppings[3]. *Salmonella typhi*, *Shigella dysenteriae* and toxigenic strains of *Escherichia coli* can be retained in the gut of cockroaches for up to several days[27].

He Fakoorziba[10], The Cockroaches hospitals in more polluted with bacteria. As a result of living habits in nutrition and the nature of the structures of their bodies and their ability to move. Adapted as to be mechanically vector for pathogens including enteric pathogens.

In hospitals, cockroaches can act as potential vectors in the epidemiology of nosocomial infections, especially the transmission of drug-resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp and several other potential pathogens[8]. Studies have shown that of the 25 different species of medically important bacteria isolated from *Blattella germanica* and *Periplaneta americana* collected from public hospitals and a residential house in central Tehran, Iran, *Klebsiella* spp was the predominant bacteria[30].

Thus, dissemination of infections is via faecal-oral route[21]. The progressive increase in antimicrobial resistance among enteric pathogens particularly *Shigella* spp, *Vibro cholerae*, Enterotoxigenic *E.coli*, is becoming critical concern of people in developing world and this is mostly related to the frequent unrestricted use of over - the - counter drugs without medical supervision[13].

Found that the control of arthropods in hospitals and homes in Morocco works to reduce pathogenic bacteria in hospitals and clinics, which reduces infection that is transmitted by Cockroach and resistant bacteria found on these insects will be related to the patient, which transmits infection and noted that the bacteria most resistance found in hospitals[28]. And also noted[15]. That cockroaches collected from hospitals and transmit the bacteria carrying

resistance to antibiotics and vectors for acquired infection. The aim of this study was to determine the Gram negative bacteria load and Multiple Antibiotic Resistance (MAR) pattern of bacteria isolated from external surfaces and internal guts of cockroaches *Periplaneta americana* collected from Haboubi Hospital in the province of Dhi Qar

Methods

1. Collection and identification of cockroaches

Eighty cockroaches were captured during the six months of the year 2015 from Haboubi hospital and placed in a sterile test tubes and transported to the laboratory and diagnosed cockroaches using standard taxonomic keys based on[1].

2. Isolation of the bacteria from external surfaces

put 2 ml of saline solution sterile and concentration of 0.85% to each tube container on the cocroaches and shake for two minutes and using a sterile pipette capacity of 1 ml worked decimal dilutions of this solution, transfer 1 ml to 9 ml normal saline, and so the transfer of 0.1 ml of which are placed on culture media and counting includes MaCconkey agar, Eosin methylene blue, blood agar and S.S agar (Oxoid , USA) By sterile pipettes category 1 ml and spread by sterile glass spreader on petri dish and placed in the incubator at a temperature 37 ° C for 24-48 hours and then colonies count isolated by colony counter system. And taking a count of the colonies were similar to Reservation a colony of one of these colonies in slant agar to diagnostic tests and placed in a temperature 5°C **[31].**

3. Isolation of bacteria in the guts

Sterilizes the outer surface by washing cockroaches in ethanol alcohol for five minutes to remove the contamination of the outer surface and dried at room temperature and washed with Normal saline for two minutes to remove the effectiveness of alcohol , and then placed cockroach on filter paper and dried also at room temperature for five minutes. And are autopsied in the laboratory within Safety cabinet after sterilization anatomy tools in alcohol are isolated swab of the bowel by loop and mixed with 2 ml of saline solution then completed itself previous procedures**[31].**

4. Total viable count

A ten-fold dilution was carried out on each suspension to determine the total viable count of each cockroach using the Spreading method counts were made on plates showing discrete colonies. A quantitative analysis of bacteria was calculated as described by[26]. The overall

load of bacteria carried by each insect from outer surface and intestine was counted and expressed as colony forming unit (c.f.u).

5. Identification of bacterial isolates

use biochemical tests Growing on Kliglar Iron agar, Catalase test ,Oxidase production Simmon citrate test , Indol test, Methyl red test ,Voges- Proskaur (VP) test , Motility test and Urease test [7] , [19] , [4] , [5] then Identification of Gram Negative bacteria. By kits API 20 E system the procedure adopted was following the manufacturer's instructions.(BioMerieux ⁄ France) was used in this study.

6. Antibiotic susceptibility testing

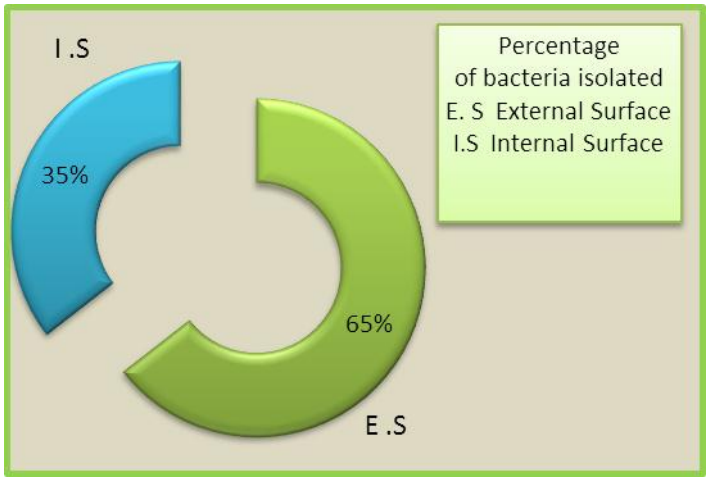
In vitro susceptibility of the bacterial isolates to twelve different antibiotics Processed from the Turkish Bio analyse company was determined using Kirby-Bauer disk-diffusion technique[2]. The turbidity of growing broth culture was adjusted with sterile broth to obtain concentration optically comparable to the 0.5 MacFarland standards tube (growth equivalent to 1.5 $X10^8$ cell/ml).The diameter of growth inhibition zones were measured by using transparent ruler. compared with the standard inhibition diameter of the CLSI (2007). The commercially available discs containing the following antibiotics:

Amoxicillin ⁄ Clavulanic acid(AMC, 30 mg), Erythromycin (E, 15 mg), Ceftazidime (CAZ, 30 mg), Ceftriaxone (CRO, 30 mg), Tetracyclin (TE,30 mg) Tobramycin (TOB,10mg) , Vancomycin (VA,30mg), Carbencilline(PY,30 mg), Piperacillin(PPL,100 mg), chloramphenicol (C, 30 mg) , Amikacin (AK,30 mg), Streptomycin (S, 10 mg).

7. Statistical analysis was done using the SPSS program version 16.0. Associations between categoric variable were tested by the chi-square test P-value < 0.05

RESULT

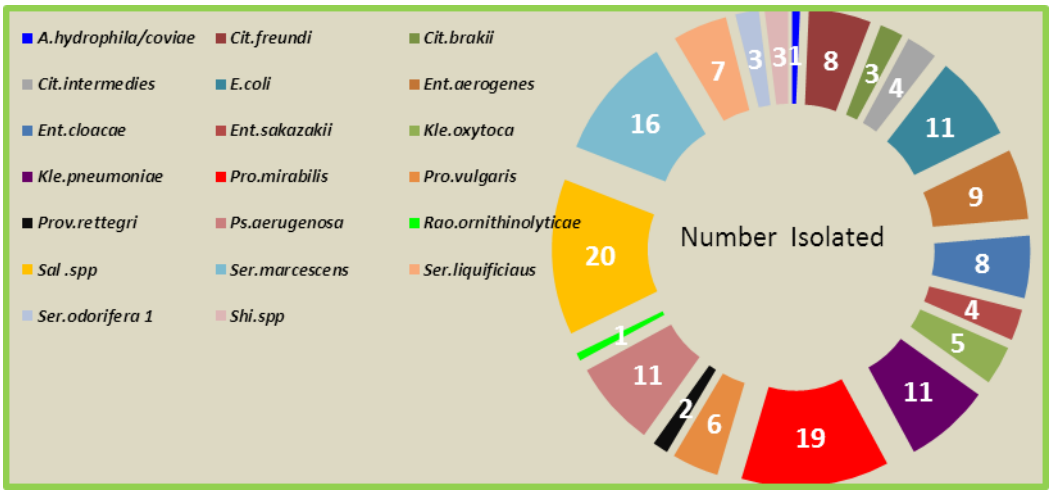
twenty (20) medically important bacterial species belonging to 11 genera were isolated from external surfaces and internal guts of eighty (80) cockroaches *Periplaneta americana* collected from the hospitals, Of the 152 bacteria isolated from the external surfaces of 98(65%) and internal guts of 54 (35 %) belonged to the group of Gram-negative bacilli (Figure 1). The variation in the occurrence of the bacteria between body surface and guts was significant ($p \leq 0.05$).



$X^2 = 9$ $df=1$

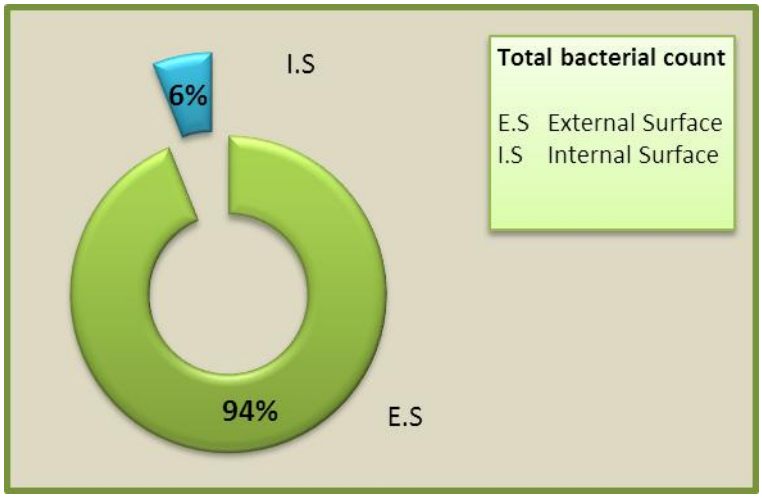
Fig 1: percentage of the Gram negative bacteria isolated from the outer surface and intestines of the Cockroaches in Haboubi Hospital

The distribution of the isolated bacteria includes the highest value to *Salmonella .Spp* (20) and *Pro.mirabilis* (19) While the lowest value of *A.hydrophila/coviae / sobria1* and *Rao.ornithinolyticae* (1) (Fig 2). Statistical analysis showed significant differences between the bacteria species isolated from Cockroaches. The level of probability ($P \leq 0.05$) .



df=19 *114.4* $X^2 =$

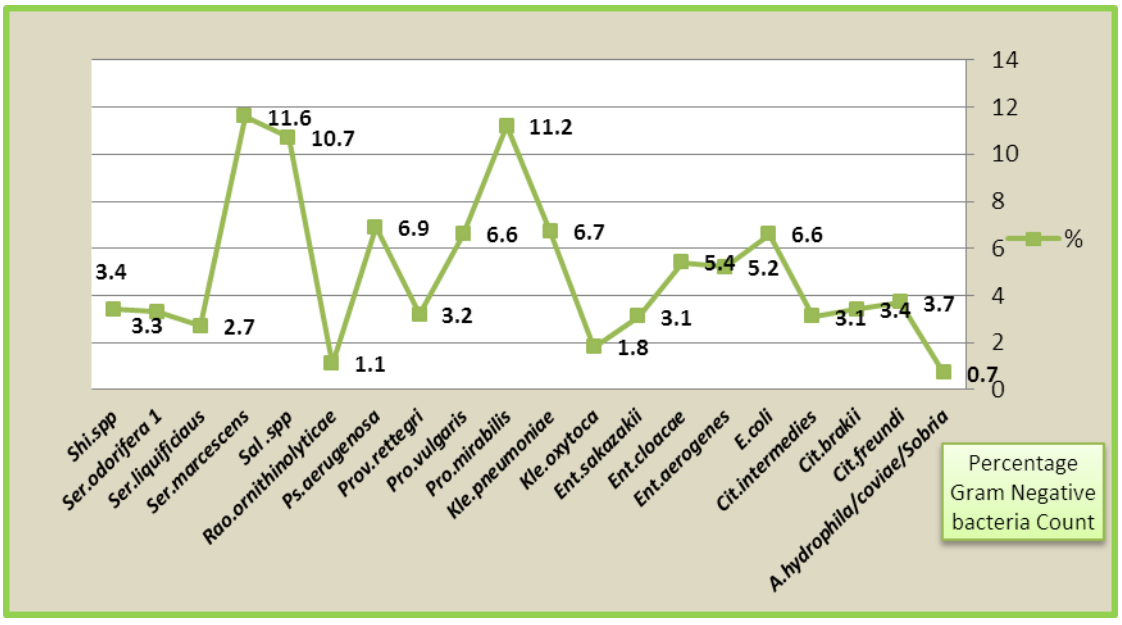
Fig: 2 Distribution of isolated Gram Negative bacteria from cockroaches in Haboubi hospital Percentage of the Gram Negative bacteria count, It reached the highest total bacterial loads on the outer surface of the Cockroaches by (94 %) While in the intestines (6 %) (Fig 3). Statistical analysis showed significant differences between the bacterial load in external surface and guts. The level of probability ($P \leq 0.05$).



X2 = 77.44 df= 1

Fig 3: Percentage of the Gram Negative bacteria count isolated from outer surface and intestines of the Cockroaches in Haboubi Hospital

Percentage Gram Negative bacterial loads isolated from Cockroches reached the highest loads to the *Ser.marcescens* (11.6%) Followed by *Pro.mirabilis* (11.2%) while less loads *A.hydrophila/coviae/Sobria* (0.7%) and *Rao.ornithinolyticae* (1.1%) Fig 4 Statistical analysis showed significant differences between the bacterial species load isolated from Cockroaches. The level of probability ($P \leq 0.05$).



X^2 =40.54 *df= 19*

Fig 4: Percentage Gram Negative bacteria count isolated from cockroaches in Haboubi hospital

The bacteria isolates have high percentage multiple antibiotic resistance ranging from 33.3 % - 100%. *Cit.brakii* have the least value of 33.3 % followed by *Cit.intermedies*, *Prov.rettegri* (50%) , while the remaining isolates demonstrated 100 % multiple antibiotic resistance patterns (Table 1). Statistical analysis showed significant differences between (MAR) pattern of the bacteria isolated from Cockroaches. level of probability ($P \leq 0.05$) .

Tab 1: Percentage incidence of Multiple Antibiotic Resistance (MAR) pattern

Isolates	Total number Of isolates	Total Number of incidence of MAR	% Frequency of MAR isolates
A.hydrophila/coviae/sobria1	1	1	100%
Cit.freundi	8	5	62%
Cit.brakii	3	1	33.30%
Cit.intermedies	4	2	50%
E.coli	11	11	100%
Ent.aerogenes	9	6	66.60%
Ent.cloacae	8	8	100%
Ent.sakazakii	4	3	75%
Kle.oxytoca	5	5	100%
Kle.pneumoniae	11	11	100%
Pro.mirabilis	19	12	63%
Pro.vulgaris	6	6	100%
Prov.rettegri	2	1	50%
Ps.aerugenosa	11	10	90.90%
Rao.ornithinolyticae	1	1	100%
Sal .spp	20	18	90%
Ser.marcescens	16	11	68.70%
Ser.liquificiaus	7	5	71.40%
Ser.odorifera 1	3	2	66.60%
Shi.spp	3	2	66.60%
$X^2 = 106.7$ *df= 19*			

The isolates demonstrated high level resistance to PY (79 %), AMC (73.6%) and CRO(73%) while VA(93.4%) was the most effective of all the antibiotics used .The pattern of antibiotics resistance of bacteria isolates were E (67.8%), S (65.8%), TE (64.5%), C (64.5%) (Table 2). Statistical analysis showed significant differences between resistance of bacteria to some of the antibiotics. The level of probability ($P \leq 0.05$).

Tab 2: Antibiotic resistance pattern of the isolates

Isolates	Total Number of isolates	AMC	E	CAZ	CRO	Te	TOB	VA	PY	PPL	C	AK	S
PHENOTYPE OF RESISTANT PATTREN													
		R	R	R	R	R	R	R	R	R	R	R	R
A.hydrophila/coviae / sobriae 1	1	1	0	1	1	1	0	1	1	1	0	0	1
Cit.freundi	8	4	2	2	3	3	3	6	5	2	5	1	2
Cit.brakii	3	2	1	1	1	0	0	2	1	1	2	0	2
Cit.intermedies	4	2	1	1	1	1	2	3	2	2	2	0	2
E.coli	11	11	9	10	11	9	3	11	11	4	9	11	5
Ent.aerogenes	9	4	3	2	3	3	3	9	4	2	9	5	6
Ent.cloacae	8	6	8	4	4	4	1	8	5	1	3	0	7
Ent.sakazakii	4	2	4	1	1	1	2	3	4	0	1	1	4
Kle.oxytoca	5	5	5	2	5	5	3	4	5	0	5	2	4
Kle.pneumoniae	11	11	11	6	11	11	8	11	11	0	11	11	11
Pro.mirabilis	19	4	10	11	19	13	12	19	14	4	11	18	19
Pro.vulgaris	6	0	2	4	4	6	3	6	4	1	3	5	6
Prov.rettegri	2	2	1	1	1	2	0	2	2	0	1	1	2
Ps.aerugenosa	11	11	9	8	9	9	3	11	10	4	10	6	8
Rao.ornithinolyticae	1	1	1	1	1	1	0	1	1	0	0	0	0
Sal .spp	20	20	19	19	20	20	10	20	20	19	20	4	10
Ser.marcescens	16	16	8	5	11	4	10	16	12	2	1	8	5
Ser.liquificiaus	7	7	5	2	3	2	5	6	4	1	2	2	2
Ser.odorifera 1	3	2	3	0	1	0	1	2	1	0	0	1	1
Shi.spp	3	1	1	2	1	3	1	1	3	1	3	0	3
%		73.6%	68%	54.6%	73%	64.5 %	46.1%	93. 4%	79 %	29.6 %	64. 5%	50%	65. 8%
X^2 = 48.3 df=11													

4. DISCUSSION

pathogenic microorganisms carried by the cockroaches collected from hospital and restaurants. Cockroaches' nocturnal and filthy habits make them ideal carriers of various pathogenic microorganisms[12]. Cockroaches feed indiscriminately on garbage and sewage and so have copious opportunity to disseminate human pathogens[21]. In this study all the *P. americana* collected from the hospitals carry various bacterial isolates of medical importance in their external surfaces and internal guts and this is in agreement with[8], [24], [11].

In this study the Gram Negative bacterial loads on the outer surface higher. Which in the guts, this high prevalence of the bacteria load harboured in the body and guts of the cockroaches portends public health risks to the people in the hospital and transmission of

nosocomial infections in the hospitals at the study area. Most of the bacterial load isolated from the cockroaches in the present study are highly pathogenic and have been implicated in many nososocomial and gastroenteric infections[29], [18].

The distribution of the isolated bacteria includes the highest value to *Salmonella. Spp* , followed *Pro.mirabilis* and *Kle. Pneumonia* The isolation of *Sal.* spp from cockroach intestine suggested them as a major reservoir of *Salmonella*.[23]. reported the presence of *Shigella* spp. in cockroaches found in hospitals indicating their importance in the dissemination of the bacterium and this study confirms it. Various bacteria were obtained in cockroaches collected from the hospitals and this shows that the hospital environments provide them with suitable temperature, humidity and food. *Kle. pneumoniae* were isolated from *P. americana* collected in the hospitals and this is in conformity with[8]. who suggested that cockroaches are possible vectors of *Kle. pneumoniae* in the hospital environment .

The bacterial isolates from *P. americana* demonstrated a multi drug resistant phenotype. Multidrug resistant *Ps.aerugenosa , Kle.pneumoniae , Sal. spp*. and *Enterobacter cloacae* with MAR index ranging from 90 – 100 % were obtained in this study. According to study[17]**.** Ps. aeruginosa is intrinsically resistant to the most commonly used antibiotics. Antibiotic resistance is achieved through a combination of restricted antibiotic uptake through the outer membrane and a variety of energy-dependent mechanisms[25]. Pre incubation with antibiotics has been demonstrated to have a number of effects on Ps. aeruginosa including induction of a biofilm form of growth, improved heat and osmotic stress response, changes to hydrophobicity, and reduced bacterial adherence[22].

Isolation of multi-drug resistant *Salmonella* spp from cockroaches has been reported by[24]. and this study confirmed it, *Cit.freundi* isolates showed resistance by 62% In this study, Approach came to study[16]. reported the occurrence of multi-drug resistant *Cit. freundii* in cockroaches.

The high prevalence of multi-drug resistant pathogens isolated from the cockroaches in hospital in the present study may be pointing to the fact there has been a prolong drug pressure/ abuse of most of these antibiotics by the doctor.

Most of the antibioticswhich had multiple resistance patterns in the isolates (such as VA, PY, AMC and CRO) were the first choice of antibiotic drugs in Haboubi hospital probably due to

the fact that they are cheap this approach to the study[9]. explain continued resistance from the misuse of antibiotics by people. and the indiscriminate use of antibiotics in the hospital . These drugs would have been seriously abused by the people due to self-medication and over-dose in Haboubi hospital While In Nigeria, the antibiotic resistance in pathogenic organisms has been reported to be plasmid mediated[20].

It is acknowledged that the focus of the this study is not geared towards investigating the mechanisms of resistance in the isolates, further studies concentrating on the mechanisms of the resistance may be of valuable information in understanding the origin of resistance observed in the isolates most importantly in hospitals .

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