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The effect of some antibiotics and antiseptics on several bacterial genera isolated from the animal house

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ABSTRACT

From different places of animal house (University of Baghdad/ biology department/ college of science), that include cages of (rats, mice and rabbits), walls, float, counters and sinks, the specimens were taken by using 25 sterile swabs. The kinds of isolated bacteria were identified according to the morphological features, biochemical tests and by VITEK2 system. fourteen strains were isolated belonged to *Pseudomonas aeruginosa*, *Pseudomonas luteola*, *Escherichia coli*, *Klebseilla pneumoniae* and *Staphylococcus aureus*. Susceptibility test were done by using VITEK 2 AST, *P. luteola* was showed sensitivity to most antibiotics but resistant to Cefazolin and Trimethoprim/ Sulfamethoxazole, but other gram negative bacteria were resistant to most antibiotics but sensitive to Gentamicin, Tobromycin, Ciprofloxacin. While gram positive bacteria (*S. aureus*) were detected as VERSA and MRSA, while sensitive to (Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Linezolid, Nitrofurantoin, Tiglycine). While MIC determination of disinfectants such as, absolute alcohol, and bleach were made for the isolated bacteria by macro-dilution method. The highest MIC value against *P. aeruginosa* from alcohol was 64 mg/ ml. while the lowest MIC value of it was against *K. pneumoniae*, *Staphylococcus aureus*. On other hand the bleach gave the higher MIC value was against *S. aureus*, *P. aeruginosa* 25 mg/ml, 16 mg/ml respectively. But the lowest MIC value against *E. coli*. We were concluded that many pathogenic bacteria can be isolated from animal house and common between humans and animals and can cause diseases to workers and researchers in animal house. They must take the necessary precautions such as sterilization of various antiseptics to avoid infection with pathogenic bacteria.

Key words: antibiotics, antiseptics, Animal house, bacteria genera.

Introduction

Laboratory biosecurity is a new concept that several laboratories around the world are also less acquainted. In regards, the term "biosecurity" applies to a protection of an animal colony from microbial contamination for those in the animal industry. laboratory biosecurity as identified by the WHO is "the safety, control and accountability of VBM (valuable biological materials) in laboratories to prevent unauthorized access, loss, theft, misuse, diversion or strategic release of VBM" (1). Laboratory biocontainment refers to the use of safety equipment and specially designed rooms and buildings which act as primary barriers to prevent the release of infectious agents, chemicals and other biological hazards into the environment (2). Although many of the microbes being examined are normally present in the environment, bacteria or viruses may not commonly have natural or enhanced virulence in the ecosystem in specific cases (3). Improvement in the prevention and treatment of infectious diseases has saved more lives than any other contribution in medical science. Clean water, immunization, antibiotic and anti-viral development were just the three main developments. We are far from overcoming the major challenge which microbes pose to human health, despite this success. All that has been achieved is the product of an increasingly sophisticated study (4). Antibiotic use and transmission of bacteria are responsible for the spread of resistant bacteria to antimicrobial agents, but their aid possible differs according to modify social and economic factors, cultural, and ecological conditions (5). In addition, there is increasing awareness of the fact that the long-term, daily use of antiseptics can generate bacterial resistance due to the exposure to sub-lethal concentrations can increase resistant to antibiotics and antiseptics, and some of species also showed cross-adaptation, as shown increase in minimum inhibitory concentration value of antibiotics and antiseptics (6), (7). The aim of study was isolation and detection of bacteria from animal house in biology department, determine the antibiotic susceptibility of isolated bacteria from animal house

by VITEK 2 system, and evaluate disinfectants effect to isolated bacteria.

Materials and Methods

Isolation and Identification of bacteria by biochemical tests:

The bacteria were isolated from the house of animals in (University of Baghdad/ Biology department/ College of Science); using 25 sterile swabs. The swabs were soaked in sterile distal water and then taken from cages of (rats, mice, rabbits), float, counters and sinks in the animal house. Bacterial diagnosis depending on the morphology of the culture samples, as well as the biochemical tests for each bacterium. The MacConkey agar was used for primary identification of gram negative bacteria; Blood agar was used in identification of isolated bacteria, and for the detection of hemolytic activity and the kind of hemolysis, EMB media and IMViC tests were used also in identification of *E. coli* from other Enterbacteriacae, and was used Cetrimide agar for *P. aeruginosa* identification, for gram positive identification was used mannitol salt agar (8). Then all inoculated media were incubated at 37℃ for overnight. And the VITEK 2 system was used for confirming the identification of bacteria.

Susceptibility test of isolates with VITEK 2 AST:

Subsequently, the strains were sub-cultivated on MacConkey agar (gram-negative) and mannitol salt agar (gram-positive) plates to test their purity and the procedure was according to company's instructions. Bacteria turbidity was also regulated with a (McFarland NO. 0.5) in sterile saline solution. VITEK 2 GNB cards, AST-No 12 cards, GPB cards. 12 cards and bacterial suspension charged the VITEK 2 system manually. Each test card was automatically filled with a bacterial suspension, sealed and incubated for 3 hours. During that time, by calculating kinetic fluorescence, the cards were read every 15 min. The samples were then marked as sensitive or resistant isolates.

MIC determination of disinfectants:

Two disinfectants (absolute alcohol, bleach (Fas / Iraq) were used in this study. The disinfectant MIC was calculated as procedure of broth dilution with macro-tube method (9). A set of test tubes with disinfectant (bleach, or alcohol) concentrations (100, 50, 25,12.5,6.25,3.125) mg/ml. Then inoculated with bacteria after dilution to the standard turbidity of McFarland (no. 0.5), after that were incubated overnight. Tubes were tested as a growth marker for turbidity changes after the incubation period. The first transparent test tube was known to be MIC.

Results and Discussion

Isolation and Identification of bacteria:

The sterile media poured in the sterile Petri-dishes, the swabs were cultured on agar media (MacConkey, blood and mannitol salt) for identification of bacteria as shows in figure (1). fourteen of them were showed growth; two isolates only were belonged to *Pseudomonas aeruginosa*, one for *Pseudomonas luteola*, four for *Escherichia coli*, two for *Klebseilla pneumoniae*. And five isolates were belonged to *Staphylococcus aureus*; the result shows in (Table 1). Buma, R. *et.al* in (2006) was reported that many types of bacteria including *Micrococcus* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus* sp., *Pseudomonas* sp., were isolated from dogs and cats' homes (10). Another study in Iraq was referred to isolate *Micrococcus* sp., *S. aureus*, *Staphylococcus epidermidis*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Pseudomonas luteola*, *Klebsiella pneumoniae* and *Escherichia coli* from internal organs of rats (11). On the other hand, study in Korea was referred to increase the drug resistance microorganisms isolated from laboratory animals never treated with antibiotics but the number of laboratory animals has increased rapidly in Korea. From total of 443 isolates (7 species) containing 5 *Sphingomonas paucimobilis*, 206 *Escherichia coli*, 60 *Staphylococcus aureus*, 15 *Staphylococcus epidermidis*, 77 *Enterococcus faecalis*, 27*Citrobacter freundii*, 35 *Acinetobacter baumannii* were isolated (12).

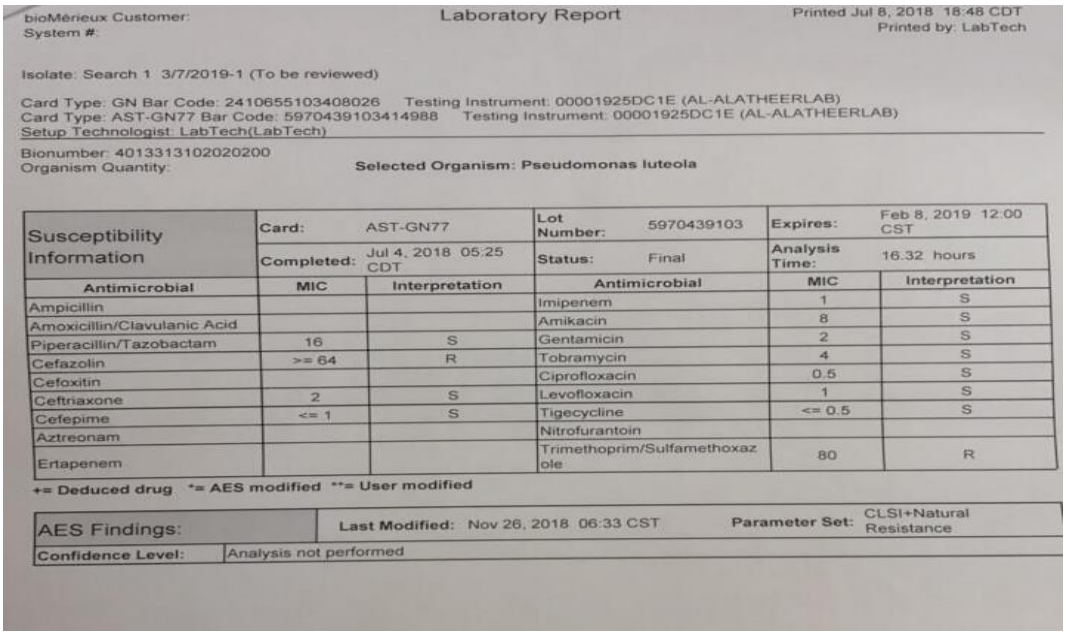
Figure (1): The isolated bacteria on media agar: A*: P. luteola* on MacConkey agar, B: *E. coli on* MacConkey agar, *C: P. aeruginosa on* Cetramide agar, *D: S. aureus* on Mannitol salt agar

Table (1): Types of isolated bacteria from Animal House in Biology Department

The place of isolation	Isolates of Gram negative	Isolates of Gram positive
Rats cages	*K. pneumoniae, E. coli, P. luteola,*	*S. aureus*
Rabbit cages	*K. pneumoniae, E. coli*	*S. aureus*
Mice cages	*E. coli, P. aeruginosa*	*S. aureus*
Counter Place of injection and dissection	*P. aeruginosa, E. coli*	*S. aureus*
Sinks	-	*S. aureus*

Susceptibility test of isolates with VITEK 2 AST:
By using VITEK AST no. 12 cards, the isolates of gram negative bacteria *(P. luteola*) was showed sensitivity to Gentamicin, Tobromycin, Ciprofloxacin, Levofloxacin, Tigecycline, Amikacin, Impenem, Cefotaxim, Pipracillin /Clavulanic acid, Cefitrixone, Cefepime and resist to Cefazolin and Trimethoprim/ Sulfamethoxazole Figure (2) , these results agree with other study which was showed that *P. luteola* was sensitive to Gentamicin, Tobromycin, Ciprofloxacin, Levofloxacin, Tigecycline, Amikacin, Impenem, ceftazidime (13). *P. aeruginosa* were sensitive to Gentamicin, Tobromycin, Ciprofloxacin, Levofloxacin, Ligecycline, Impenem and resistant to other antibiotics. *K. pneumoniae* isolates were sensitive only to Gentamcin, Tobromycin, Ciprofloxacin, Impenem. But *E. coli* resistant to Impenem, Cefotaxim, Pipracillin /Clavulanic acid, Cefitrixone, Cefepime, Cefazolin. While gram positive bacteria (*S. aureus*) were detected as VERSA

and MRSA, and they were resisted to Vancomyin, Cefoxitin, Erythromycin, Clindamycin, Oxacillin, Tetracycline, Fusidic acid, Teicoplanin, Rifampicin, while sensitive to Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Linezolid, Nitrofurantoin, Tiglycine. The isolated bacteria *Staphylococcus sp.*, *E coil*, *Proteus sp.* and *Pseudomonas sp.* from the dogs and cats were resistant to Lincomycin, Vancomycin hydrochloride, Ampicillin, Trimethoprim/ Sulfamethoxazole and Tetracycline (11), while Bao, L. *et al* in (2013). It was confirmed that the *Klebsiella pneumoniae* clinical isolate was susceptible to Amikacin, Cefepime and Imipenem, when it was highly ampicillin resistant. *Pseudomonas aeruginosa* was susceptible to Cefepime and Imipenem while high resistance to Cefoperazone / Tazobactam and Cefotaxime was observed. Most of the antibiotics were resisted to *Acinetobacter baumannii* and *Escherichia coli* (14). In addition, resistance of bacteria isolated from stool of human, livestock, dogs, wildlife and water sources were resisted to ampicillin, streptomycin, sulfamethoxazole, trimethoprim, and tetracycline across livestock types was higher (>35% in Arusha, >30% in Maasai, and >10% in Chagga) compared to ceftazidime, chloramphenicol, ciprofloxacin, and kanamycin in Tanzania (5). The use of antibiotics in accrue of small mammals, with specific antibiotics was also challenge because increased the resistant to antibiotics in all the world. But in this study was used VITEK 2 AST to know the sensitivity test to all bacteria isolated from animal house, which can transport to workers and researcher in these places.



bioMérieux Customer:
System #:

Laboratory Report

Printed Jul 8, 2018 18:48 CDT
Printed by: LabTech

Isolate: Search 1 3/7/2019-1 (To be reviewed)

Card Type: GN Bar Code: 2410655103408026 Testing Instrument: 00001925DC1E (AL-ALATHEERLAB)
Card Type: AST-GN77 Bar Code: 5970439103414988 Testing Instrument: 00001925DC1E (AL-ALATHEERLAB)
Setup Technologist: LabTech(LabTech)

Bionumber: 4013313102020200
Organism Quantity:

Selected Organism: Pseudomonas luteola

Susceptibility Information	Card: AST-GN77		Lot Number: 5970439103	Expires: Feb 8, 2019 12:00 CST	
	Completed: Jul 4, 2018 05:25 CDT		Status: Final	Analysis Time: 16.32 hours	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin			Imipenem	1	S
Amoxicillin/Clavulanic Acid			Amikacin	8	S
Piperacillin/Tazobactam	16	S	Gentamicin	2	S
Cefazolin	>= 64	R	Tobramycin	4	S
Cefoxitin			Ciprofloxacin	0.5	S
Ceftriaxone	2	S	Levofloxacin	1	S
Cefepime	<= 1	S	Tigecycline	<= 0.5	S
Aztreonam			Nitrofurantoin		
Ertapenem			Trimethoprim/Sulfamethoxazole	80	R

+= Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Nov 26, 2018 06:33 CST	Parameter Set: CLSI+Natural Resistance
Confidence Level:	Analysis not performed	

Figure (2): The result of Susceptibility test by VITEK 2 AST for *P. luteola*

MIC determination of disinfects:

The results of MIC of two disinfectants (alcohol 96%, bleach) against the isolated bacteria are showed in Figure (3). The highest MIC value against *P. aeruginosa* from alcohol was 64 mg/ ml. while the lowest MIC value of it was 6.25 mg/ml against *K. pneumonia, Staphylococcus aureus.* On other hand the bleach gave the higher MIC value was against *Staphylococcus aureus, P. aeruginosa* 25 mg/ml, 16 mg/ml respectively. But the lowest MIC value against *E. coli*. Buma, R. *et.al*.(2006) was examined the activity of two liquid hand soap as antimicrobial agent, Liquid hand soap containing triclosan and triclocarban but the other contain isopropyl methyphenol were effective against different types of bacteria with different range of dilutions (10).while in another study was found that ethanol present antimicrobial activity, with decrease populations of *B. stearothermophilus, B. subtilis, S. aureus and E. cloacae* it was necessary a MIC of 87.5mg/ml, while *E. coli* presented a MIC of 65.650 mg/ml. *S. marcescens and A. calcoaceticus* presented higher sensitivity, with MIC 43.750 mg/ml (15). Cleaning has resulted in a further reduction of total aerobic bacteria, all studies were showed that a disinfection stage continues to be an effective stage in reducing the risk of bacterial infection in barns with bacteria in high level (16). In another study allowed determining the MIC of the ethanol extract on the reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 on wound of rats. 10 mg/ml is the concentration that inhibited the growth of *E. coli* ATCC 25922. The MIC obtained for the reference strain *S. aureus* ATCC 25923 is 0,078 mg/ml (17).

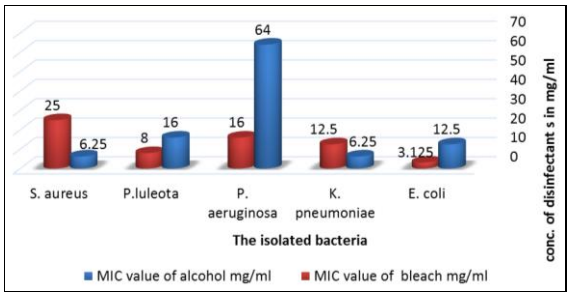


Figure (3): The MIC Values of Alcohol and Bleach against Isolated Bacteria from animal house

Conclusions

By this study was isolated many genus of bacteria from animal house. And these bacteria can transfer from animal to human, therefore; they dangerous to human which they worked in animal house and cause many diseases to these groups of people. But these bacteria could be treated or killed with antibiotics and disinfectants like alcohol and bleach. On another hand the resistant of these bacteria can be increased to antibiotics and become problem to Workers or researchers who deal with laboratory animals must take the necessary precautions such as sterilization of various antiseptics to avoid infection.

Conflicts of interest

No conflicts exist.

Acknowledgment

This research was conducted with the assistance of the Department of biology in the College of Sciences, University of Baghdad.

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تأثير بعض المضادات الحيوية والمطهرات على العديد من الأجناس البكتيرية المعزولة من البيت الحيواني

ايناس غسان سويدان ، سعاد علي احمد ، حسام محمود حسن

الملخص

من اماكن مختلفة في البيت الحيواني التابع لقسم علوم الحياة/كلية العلوم/جامعة بغداد والمتضمنة اقفاص (الجرذان،الفئران،الارانب)،الجدران، الارضية، البينجات و المغسلة ، تم اخذ 25 عينة بواسطة مسحات معقمة. شخصت انواع البكتريا المعزولة تبعا لمظهرها الخارجي، الاختبارات الكيموحيوية و باستخدام جهاز VITEK 2 system . تم عزل 14 عزلة بكتيرية تعود ل *Pseudomonas aeruginosa, Pseudomonas luteola, Escherichia coli, Klebseilla pneumoniae ،Staphylococcus aureus* . اجري اختبار الحساسية لانواع البكتريا المعزولة باستخدام VITEK 2 AST، *P. luteola* اظهرت حساسية تجاه معظم المضادات الحيوية لكن مقاومة للسيفازولين و الترايميثوبريم /سلفاميثازول، لكن البكتريا السالبة لصبغة كرام كانت مقاومة لمعظم المضادات وحساسة (للجنتامايسين، التوبرامايسين، السبروفلوكساسين)، بينما البكتريا الموجبة لصبغة كرام (*S. aureus*) شخصت MRSA VERSA و كانت حساسة (للجنتامايسين، التوبرامايسين، الليفوفلوكساسين، موكسيفلوكساسين، اللنيزولد، النتيروفيورانتيون و التايكلايسين). وتم تحديد التركيز المثبط الادنى للعزلات باستخدام انواع من المطهرات مثل الكحول المطلق والقاصر. اعلى قيمة للتركيز المثبط الادنى 6.25 mg/ml باستخدام الكحول كانت ضد *P. aeruginosa* ، بينما كانت اقل قيمة له ضد *K. pneumoniae, S. aureus* من جانب اخر كانت اعلى قيمة للتركيز المثبط الادنى باستخدام القاصرضد *S. aureus, P. aeruginosa* و تساوي 25 mg/ml, 16 mg/ml على التوالي. في حين اقل قيمة له كانت ضد *E. coli*. نستنتج مما سبق ان عددا من البكتريا الممرضة ممكن عزلها من البيت الحيواني والتي تكون قادرة على الانتقال بين الانسان والحيوان وممكن ان تسبب امراض مختلفة للعاملين والباحثين في البيت الحيواني، يجب عليهم اتخاذ الاحتياطات اللازمة مثل استخدام مواد التعقيم المختلفة لتجنب العدوى بالبكتريا الممرضة .

الكلمات المفتاحية: المضادات ، المطهرات، البيت الحيواني ، الاجناس البكتيرية.