Typing of *Acinetobacter baumannii* Isolates from some Iraqi Children infected with Head Lice

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Introduction

Head lice invasion a worldwide health care problem, which obligatory human hematophagous ectoparasites belong to pediculide family, it caused by pediculus humans capitis [1] in fact the USA was recorded 6-12 million of head lice invasion appeared every year [2]. Children with head lice usually related with negative feeling additional to negative consequences as quarantine, Furthermore increase resistance of lice to treatment with neurotoxic pediculicides, Meta-methrin one of the neurotoxic insecticide shampoo which is used to executive head lice, head lice removing are getting scarcely to cure despite general home clear out procedures.

Furthermore, many studies have been shown the lice resistance to many of commonly applied pediculicides [3]. The evidence of the ability of lice to transmit some of the human infectious agents such as Rickettsia prowazekii, Bartonella Quintana and Acinetobacter baumannii [4].

Acinetobacter baumannii called the Iraqi bacter, A comparatively benign bug becomes an extremely lethal pathogen, known to U.S. soldiers as Iraqibacter after 2003 war in Iraq [5]. A. baumannii has enduring to be a terrible problem for veterans, researches in environment and soldiers who served in Iraq [6]. A. baumannii also the significant member of ESKAPE pathogen (E. faecium, *Staph. aureus*, K. pneumoniae, A. baumannii, *P. aeruginosa*, and Enterobacter species), a group of bacterial pathogens with a elevated velocity of antibiotic resistance that are responsible for the preponderance of A hospital-acquired infection (HAI) [7]. It is still an opportunistic human pathogen, which causes life-threatening nosocomial infections such as ventilator-associated pneumonia, meningitis, urinary tract, bacteremia and wound infections.

A. baumannii harboring a number of effective virulence factors, which painstaking an organism of low virulence, but the occurrence of fulminant community-acquired Acinetobacter pneumonia, indicates that these organisms possibly sometimes shows high pathogenicity and cause invasive disease. These factors embrace the connection and perseverance on dry and/or solid surfaces, the capability to take necessary nutrients such as iron, the adhesion to and succeeding destroying of epithelial cells, and the capacity in some strains to produce gelatinases and proteinases that injure host tissues [6].

Dissimilar to another type of the Acinetobacter genus, which are often in
accessible from the water, soil and animals [8], A. The bunny is almost completely found in the hospital setting, causing (HAI). However, environmental incidence had been notified in not many cases according to [9]. The highest occurrence of A. baumannii in non-clinical sources appears to be associated with lice [5-10]. Other non-human sources include animals, with several strains isolated from cases of infection of cats, dogs and horses [11], and colonization of fish, poultry and slaughterhouse meat [12].

A. baumannii can also found in aquaculture and in soils [13-14], on human skin [15]. In remains to were estimated if these isolates, which are infrequent, are the result of hospital contamination or originate from natural reservoirs of this species.

**Material and Methods**

One hundred twenty children infected with lice in two Kindergartens (Al-Safa Kindergarten and Al-Rayahin Kindergarten) in Al-Rusafa, Baghdad, Iraq. These children with ages 3-6 years included 93 girls and 27 boys. These lice and egg of lice collected from a hair and skin of infected children (which show in figure 1). The sample was rinsed into a vial contains phosphate-buffered saline solution (0.15 M, pH 7.3) and transport media without any solidifying agents then, sent to the laboratory of microbiology department (college of science /Al-Mustansiriya University). Each sample was compressed slowly with sterilized glass rood in phosphate buffer saline, suspend well, then 0.1 ml of final suspension was transferred to brain heart infusion broth directly incubated for 24h at 37°C. The specimens (cultured and swabs) were inoculated onto appropriate plates for standard aerobic and microaerophilic bacteria (5-10% of co2) cultures were incubated at 37°C for 24 h, 48 h respectively.

The positive cultured samples were tested for the presence of different aerobic bacteria, microaerophilic bacteria and Candida species by general culture morphology, biochemical test and germ tube test for Candida species. The isolated pathogens were identified by using the automated system VITEK 2 (Bio Merieux, Marcy L’ Etoile, France). Furthermore, some of these lice put it in formalin 10% for electronic microscope study.

**Genotype Diagnosis of A. baumannii by PCR**

RecAgene (a house keeping gene) was used for A. baumannii genotypic diagnosis. Specific primer listed in table 1 had been employed and the amplified size was 240bp. Template DNA was prepared by the boiling method by [16]. Briefly, few isolated colonies of overnight growth bacteria had suspended thoroughly in (1mL) distilled water and boiled in a water bath for (10min). After centrifugation, supernatant had been used as template DNA in PCR technique. Moreover confirmed five isolates A. baumannii genetically by using rec Agene as a housekeeping gene as it appeared in the Table (1).

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Figure 1: Head lice collected from infected children, F: show the cheese like accumulation of Candida albicans, I & M: show irritation, redness of lice bite
Table 1: The primers used in the current study for PCR amplification

<table>
<thead>
<tr>
<th>Name of Genes</th>
<th>Primers 5' - 3'</th>
<th>Size products</th>
<th>Tm</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RecA</td>
<td>F: CCTGAATTCATTCGGGTCGAAC&lt;br&gt;R: GCTTTGTGCTGCAACAAC</td>
<td>425</td>
<td>54</td>
<td>17</td>
</tr>
<tr>
<td>RAPD</td>
<td>5' - ACGGCGGACC-3'</td>
<td>-</td>
<td>44</td>
<td>18</td>
</tr>
</tbody>
</table>

Antimicrobial Susceptibility Testing

It had performed by the disk diffusion method according to the CLSI guidelines 2016[19]. The following disc (μg/disc). Ak: amikacin (30 μg), GN: gentamicin (30 μg), IMI: imipenem (10 μg), NOR: Norfloxacin (10μg), ATH: Azithromycin (15 μg), LEV: Levofoxacin (5 μg), AUG: Amoxicillin + Clavulanic acid (30μg). MDR was defined as resistance to three or more antibiotics of the following classes: quinolones (ciprofloxacine), aminoglycosides (gentamicin), extended spectrum cephalosporins and carbapenems (imipenem) (20).

The Minim inhibitory concentration MIC was done by using the agar dilution method to determine the break point of meta-methrin insecticidal effect on Acinetobacter baumanii, and using quality controlled standard strains (Acinetobacter baumanii ATCC BAA-747) obtained from the American Type Culture Collection.

Virulence Factor Detection Assays

Virulence factor phenotypic detection of A. baumannii isolates was done in order to detect for the ability of biofilm formation and other ten virulence factors. Which were (Capsule, Motility, Twitching motility, Hemolysin, Pellicle, Protease, Lipase, Lecithenase, Gelatinase and Sidrophore) [21-22].

PCR and Clonal Diversity Analysis

The clonality of A. baumannii isolates was determined using the RAPID-PCR method this primer for “random amplification of polymorphic DNA” as seen in (table 1). Cycling conditions for RAPID-PCR are as follows: 45 cycles of 1 min each at 94°C, 44°C, and 72°C. After the last cycle, samples had maintained at 72°C for 10 min. The PCR products were analyzed by horizontal electrophoresis according to Sam brook and Russell (23) by using of (15μl) of the reaction product on a 1% agarose gel and 10 kb DNA ladder (Kapa, South Africa) as a molecular marker, then PCR products were visualized with UV light at 336 nm. The Numerical Taxonomy System 1.8 software, using the Jaccard coefficient of similarity, and the unweighted pair-group method with arithmetic mean (UPGMA) had used in calculating genetic distance and obtaining a phylogenetic tree.

Results

The results of this study showed 93 (77%) girls infected while 27(22%) boys in head lice. The positive cultures results from both samples type's swabs and crushed lice showed the diversity of bacterial isolates in additions to the Candida albicans, these isolates were: 3 isolates Candida albicans, 11 isolates Staph. sp., 3 isolates Pseudomonas aeruginosa, 1 isolate of E. coli, 1 isolate of Klebsiella pneumonia and 5 isolates A. baumannii, which appear in Figure (2) All A. baumannii was confirmed the diagnosis with used recA gene (housekeeping gene).

Figure 2: Distribution of bacterial isolates Candida albicans, Staphylococcus spp., klebsiella, pseudomonas, E.coli and A. baumannii from Lice
While Figure (3) shows the resistance percentage profile towards antibiotics used in this study, these percentages diverge completely from resistance towards CRO was reached 100% for GN, LEV and CD, followed by 80% for CEF, ATH, NOR, DO and CLR.

The results of MIC have been exposed the capability of all \textit{A. baumannii} isolated to grow in concentration 2%, 3%, 4%, 5% of meta-methrin while only one isolate can grow in 6% concentration of meta-methrin. Furthermore, the detection of virulence factor have been revealed that each isolate have more than one virulence factor as seen in the Table (2) and figure (4), all \textit{A. baumannii} isolates non-biofilm producer, nonmotile and negative in gelatinase and protease reactions. Otherwise, all isolates were capsulated and found to be positive in twitching motility, pellicle producer but only one isolate found to be positive in Lecithinase, Siderophore production and \(\beta\)-hemolysis on blood agar.

### Table 2: The results of virulence factors detection assays for \textit{A. baumannii} Lice isolates

<table>
<thead>
<tr>
<th>test No.</th>
<th>Capsule</th>
<th>Motility</th>
<th>Twitching motility</th>
<th>Hemolysin</th>
<th>Biofilm</th>
<th>Pellicle</th>
<th>Protease</th>
<th>Lipase</th>
<th>Lecithinase</th>
<th>Gelatinase</th>
<th>Siderophore</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>B</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
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<td>+</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td>Y</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 3: Antibiogram profile of Acinetobacter baumannii isolates

Figure 4: Positive and negative results of some virulence factors detection assays for \textit{A. baumannii} Lice isolates. A: Biofilm test. B & C: Protease assay (Caseinase), D: Lecithinase assay, E: Motility and twitching motility assay, F: Siderophore produce assay, G: Gelatinase test, H: Hemolysin test.
The results of random amplification of polymorphic DNA (RAPD-PCR) were showed each A. baumannii isolates isolated from lice had specific genetic profile as shown in Figure (5), while genetic segregation of isolates depending on phylogenetic trees appeared in Figures (6,7,8).

Figure 5: RAPD fingerprint profile of A. baumannii isolates. Agarose gel electrophoresis had performed using 1% agarose gel, and the run lasted for 90 min at 70 V.

Figure 6: Dendrogram of A. baumannii by APGMA jaccard according to antibiotics resistant

Figure 7: dendogram of A. baumannii by APGMA jaccard according to virulence factors

Figure 8: Dendrogram of A. baumannii by APGMA jaccard according to RAPD-PCR
Discussion

The outcome of the current search proved high percentage of infected girls compared with infected boys, according to many endemic regions, the girls is more effected by head lice than boys the ratio appear in Australia 2:1 while in turkey 12:1, there is belong to gender-typical behaviors, long hair of girls and it is not repercussion any biological determined that increased susceptibility in girls [24].

The cultures of crused lice and swabs were showed more than one type of bacterial isolate, moreover confirmed present A. baumannii isolates in this study was agree with (25) which refer to the percentage of A. baumannii isolated from human lice was 58% Rwanda, 35% peru and 18% in France. The improve of present of A. baumannii very effective by using PCR technique as recorded by (26). Furthermore, some of S. aureus and Streptococci isolates can cause secondary infection belong to the crushed of skin (24).

A. baumannii, which is widespread in nature (water, soil, living organisms, vegetables, and the skin of patients and healthy subjects). An increase in the frequency of community-acquired infections with A. baumannii had recorded during the last decade, mainly from countries located in inter-tropical areas or during international armed conflicts or natural disasters, which raises the query of a probable environmental reservoir. Our study found that 5 isolates of A. baumannii (3 from lice collected from girls one from skin girl swab beside one Klebsielle and one Staph. spp isolate and one from lice collected from boy beside one isolate of Staph. spp) of hair lice collected wide-reaching was naturally infected.

Until now, it is still unknown how all these hair lice acquire their A. baumannii infections and other bacteria, which isolated (5) but perhaps the association of A. baumannii with lice belong to undiagnosed bactermiain patients harboring with lice. In animals and arthropods (lice) infection due to Acinetobacter sp. is consider as an emerging problem due to escalating in the number of reports from different countries across the global.

The results of antibiotics susceptibility teste showed the high level of resistance against almost antibiotics make it a multidrug resistance bacteria which maybe use to treat this infection if occurs in human specially these isolates isolated from children, and maybe interrupted any invasion or injury even necrosis in human skin and cause bacteremia or other terrible problem (27). The ability of A. baumannii isolates to grow in meta-methrin was appeared in MIC test, infact the dose was used in children shampoo range from 2-4% therefore the treatment appear usefulness to treated this bacteria which found in lice or the infected skin. As well as the virulence factors plays asignificant role in resistance to defense mechanisms such as leukocytes, antibodies, and complement which found in blood meal of those lice (24).

Moreover, A. baumannii able to cause a wide-range of infections, is increasingly resistant to antibiotics and may be associated with high mortality rates in different patients, its virulence factors, pathogenicity mechanisms were largely unknown, and genetics study still limited at this time for this bacteria (28). However, we keep this isolates to study different molecular mechanisms of virulence factor, Quorum sensing, and Pathogenicity Island in A. baumannii from lice. While still all study in my country and across the global in virulence factor is limited because eight completed and annotated genome sequences of A. baumannii are now available, and about 60 others are in progress according to (24) in Italy. Also no study these factor in molecular level or show increase or inhabit these factor also no biotechnology application study of isolates from lice and no study in Iraq isolate or diagnosis bacterial head lice.

The results of random impification of polymorphic DNA (RAPD-PCR) were showed each A. baumannii isolates isolated from lice had specific gene profile gave it special DNA finger print as seen in Figure (5), in fact, various gene-typing methods, like pulsed-field gel electrophoresis (PFGE), restriction fragment length polymorphism analysis, RAPD-PCR, had been used for the typing of strains from diverse sources. Genetic typing methods had been found to be more discriminatory than phenotypic methods for typing A. baumannii isolates (26, 30). RAPD does not require any specific knowledge of the DNA sequence of the target organism. The single short primer will amplify a sequence of DNA, depending on positions that are complementary to the sequence of a primer.

A baumannii is among the most disconcerting pathogens and has been implicated in several types of nosocomial infections due to its
seemingly endless capacity to acquire antimicrobial resistance mechanisms. In addition to its ability to acquire substantial resistance genes, its remarkable ability to adjust itself in the hospital environment, thus further enhancing its widespread transmissibility had resulted in A. baumannii becoming a leading pathogen of nosocomial infection [28]. In 2006, the genome of A. baumannii strain isolated from a human body louse had sequenced and analyzed; results showed that this remarkably susceptible strain harbored several hundred-insertion sequence elements (transposons, gene cassettes from class 1 integrons), with gene cassettes (sometimes chimeric) mostly originating from the genera Pseudomonas, Salmonella, and Escherichia.

Together with A. baumannii, many members of these genera are commonly found in aqueous environments of healthcare facilities, where, under antimicrobial pressure in these settings, genetic exchange among them may be promoted) that have played a crucial role in its genome reduction (5, 31). The clonal diversity by APGAM jaccard was showed two major groups of isolates furthermore the relatedness between isolates which appeared isolated 4 and 5 more similar 0.83 distance while isolates 2, 4,5 descended from the same group as appeared in figure (6).

On the other hands the dendrogram of virulence factors was showed two major groups one of them was contained isolate 1 and the other group showed high similarity 1.0 between 3, 4, 5 isolates as it appeared in figure (7), while the dendrogram of profile genes appeared the relatedness between 3,4 isolated was 0.75 distance and gave view of diversity between isolated despite the similarity according to the antibiotics resistance and virulence factors. The results of this study were revealed isolate 1 have multi-virulence factors additional to multidrug resistance and special DNA fingerprinting making it in a special group in dendrograms.

Conclusion
Our study is the first showing the presence of A. baumannii in human head lice in children, in Iraq, especially this bacteria is a very adaptable pathogen, with an extraordinary ability to acquire new genetic material, beside other pathogenic bacterial species and the effect of meta-methrin on bacteria. In addition, one isolate of A. baumannii no.1 was epidemic clonal lineages show multifactorial and combinatorial traits and particularly, resistance to different antibiotics, could have favored the spread and persistence among children in Kindergartens and also may be transmitted to them families and another member in them society with head lice and causing epidemic with A. baumannii.

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