Identification of the most bacterial isolates from the bile of broiler Chicks by API 20E system

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Abstract

This study was coducted in Thi-Qar Governorate from January 2009 through March 2009. Seventy five samples of the gallbladder were collected randomly from broiler chicks in a sterile containers .

All samples were cultured on blood and MacConkey agar for bacterial isolation. The pure isolates were identified by the API 20E system. This study revealed that the most common bacterial isolates which resist and survive the bile constituents was *Klebsiella pneumoniae* (60%) which was isolated from (45) samples followed by *Proteus mirabilis* which isolated from (20) samples (26.6%). Where as there was no any other isolates from the other samples (10)which represented to (13.4%).

Introduction:

Bile is one of many barriers that some bacterial species overcome in the gastrointestinal tract inorder to infect and cause disease (Begley and Hill, 2002).

During infection, pathogen must sense and respond to harsh environment with the host such as small intestine and gallbladder, Bile found in these two environments and consisting primarly of bile salts, degrads and disperses lipid during digestion and , such as , is a potent antimicrobial(Gunn,2000).

A large portion of the work in a diagnostic laboratory consist the identification of bacterial pathogens from the family *Enterobacteriaceae*.

Veterinary diagnostic laboratory identifications generally rely on conventional tube media and lack the standardization provided by rapid microidentification system .These micro test systems are designed for easier use and provid more rapid and accurate identifications (Smith etal., 1972).

***Klebsiella pneumoniae* is a facultatively anaerobic gram negative bacterium, which is a miner intestinal commensal organism. This bacterium ,however, is an opportunistic pathogen that has been implicated in case of mastitis in cattle, metritis in mares, bacteremia in calves, and urinary tract infections in dogs, pneumonia and septicemia in foals (Wilson and Madigam,1989), and polyarthritis in kids (Bernabe *etal.*,1998). Additionally , it is an important cause of nosocomial wound and urinary tract infections of hospitalized humans and animals (Glickman,1981).**

***Proteus mirabilis* is one of the most frequent etiological agents associated with urinary tract infections , it is amember of the normal flora of the mammalian intestinal tract and has been isolated from humans and different animals (Guentzel,1995).Additionaly it is a widely distributed in the environment, occurring in polluted water and soil , where it play an important role in decomposing organic matter of animal origin (Rozalski and Sidorczy,1997).**

The objective of this study were to identify these organisms by using of API 20E system (Analytic profile index and complementory computer survice) which was a test kit for the identification of enteric bacteria provides an easy way to inoculate and read test releavent to members of the family *Enterobacteriaceae* (Holmes *etal*.,1978).

This work further supports evidence that bile was an important environment signal for enteric organisms and that even closely related enteric organisms have developed unique pathways to utilize bile as ahost derived signal (Prouty *etal.*,2004).

Materials and Methods:

Seventy five gallbladder samples were collected from broiler chicks in different areas in Thi-Qar Governorate . Bile was aspirate by sterile syringe , then each samples was streaked on the blood and MacConkey agar plates. The inoculated plates were inoculated at 37C° for 24-48 hours .

Identification of pure colonies were done according to microscopic examination and API 20E system .The API 20E test kit (biomerieux,Marcy-1 Etoile, france) aplastic strip holding twenty mini-tubes was inoculated with a saline suspension of pure culture (as per manufactures directions).

This process also rehydrates the dessicated medium in each tube, A few tubes are completely filled (CIT,VP and GEL), and some tubes are overlaid with mineral oil such that anaerobic reactions can be carried out (ADH, LDC,ODC,H2S and URE).

After incubation in a humidity chamber for 18-24 hours at 37C° the colour reaction was read (some with aid of added reagents)and the reaction plus the oxidase reaction done separately were converted to a seven – digit code .This code is fed in to the manufacturers database or index which gives back the identification ,usually as genus and species .

Biochemical tests of API 20E system

Test	Substrate	Enzyme reaction	Positive result	Negative result
ONPG	Orthonitrophenol glycoside	B-galactosidase	Yellow	colorless
ADH	Arginine	Arginine dihydrolase	Red-orange	Yellow
LDC	Lysine	Lysine dicaroxylase	Orange	Yellow
ODC	Ornithine	Ornithine dicaroxylase	Red-orange	Yellow
CIT	Soduim citrate	Citrate utilization	Greenish-blue green	Green-yellow
H2S	Soduim thiosulfate	H2S production	Black	Colorless-gray
URE	Urea	Urease production	Red-orange	Yellow
TDA	Tryptophane	Tryptophane deaminase	Red-orange	Yellow
IND	Tryptophane	Indole production	Red ring	Yellow ring
VP	Soduim pyruvate	Actone production	Pink-red	Colorless
GEL	Gelatin	Gelatinase	Change in color	Black
GIU	Glucose	O/F	Yellow	Greenish blue- blue
MAN	Mannitol	O/F	Yellow	Greenish blue- blue
INO	Inositol	O/F	Yellow	Greenish blue- blue
SOR	Sorbitol	O/F	Yellow	Greenish blue- blue
RHA	Rhaminose	O/F	Yellow	Greenish blue- blue
SAC	Sucrose	O/F	Yellow	Greenish blue- blue
MEL	Melibiose	O/F	Yellow	Greenish blue- blue
AML	Amygdalin	O/F	Yellow	Greenish blue- blue
ARA	Arabinose	O/F	Yellow	Greenish blue- blue

Results:

Laboratory diagnostic methods showed that there were (45) samples (60%) related to *Klebsiella pneumoniae* (Table 1), and 20 samples (26.6) related to *Proteus* spp. (Table 2) which finally diagnosed by API 20E system as *Proteus mirsbils* .

***Klebsiella pneumoniae* was diagnosed by the API 20E system (Figure 1) in fourty five of seventy five bile samples obtained from broiler chicks (60%)as the most common bacterial isolate from the gallbladder of these chicks , while the *Proteus mirabilis* was diagnosed (Figure 2) in twenty of seventy five samples (26.6%),where as there was no any other isolates from the other samples (10),which represented to (13.4%) (Table 3).**

Table 1 :Laboratory diagnostic Characters of *Klebsiella pneumoniae*

Laboratory diagnostic method	Characters
Microscopic examination	Gram negative rod ,capsulated, non-motile .
On blood agar	Large,rounded,mucoid colonies
On MacConkey agar	Large,rounded,mucoid colonies slightly elevated, pink(lactose ferminter).

Table 2: Laboratory diagnostic Characters of *Proteus* Spp.

Laboratory diagnostic method	Characteristics
Microscopic examination	Gram negative rod,motile
On blood agar	Spread on the surface of plate (swarming),non- hemolytic.
On MacConkey agar	Small,rounded colonies,pale(lactose non-ferminter)

Table 3 : The number and the ratio of the bacterial isolates in broiler chicks Gallbladder.

Bacterial isolates	Number	Ratio
Klebsiella pneumoniae	45	60%
Proteus mirabilis	20	26.6%
No isolate	10	13.4%

Figure (1)

Identification of *Klebsilla pneumoniae*
by API 20E system

Figure (2)

Identification of *Proteus mirabilis*
by API 20E system

Discussion:

Evolution of bile resistance mechanisms in gram negative bacterial isolates is an unavoidable phenomenon because it represents anatural adaptive response to environmental stress (Hasdemir *etal.*,2004).

Some workers consider it desirable to evaluate the action of bile on bacterial growth in terms of stimulation , no effect , or inhibition (Shimada *etal*.,1970).

In this study *Klebsiella pneumoniae* and in alower degree *Proteus mirabilis* (Table 1) have been found not to be limited to intestinal tract and have been recovered in significant incidence in the gallbladder of commercial broiler chicks. This mean that there is a relationship between bacteria and bile that makes these bacteria can resist the bile components and survive in the internal tissues such as the gallbladder (Begley and Hill, 2005).

Neumerous studies in recent years have been undertaken to determined where inside the body of the birds these organisms are located and try to determined the significance of these reservoirs in contamination of poultry flocks(Cox *etal*., 2007).

The significance of these reservoirs in the internal organs of commercial broilers is yet to be determined ,but can play a role in the microbiology of the intestinal tract and hence the final food product (Dewit *etal*.,1979).

Methodology procedure utilized in this study has been shown to select for this type of species and excluded other species from being detected(Daniele *etal*.,2006).

This system appears to be capable of identifying veterinary bacteria in the family *Enterobacteriaceae* with a high degree of accuracy ,also it is able to identify pathogens with equal or better accuracy as compared to conventional methods used by most diagnostic laboratories .It is also advantageous in that it is a very standardized system .The stip is always inoculated in the same manner, it contains a standard set of twenty biochemical tests (Robertson *etal*., 1976).

Overall, the API identifications were more accurate than the diagnostic laboratories identifications, it correctly identified sixty five of the veterinary isolates from the bile of broiler chicks (Rutherford *etal.*, 1977).

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تمييز اهم العزلات البكتيرية المعزولة من صفراء فروج اللحم باستخدام نظام API 20E

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الخلاصة

تم أجراء هذه الدراسة في محافظة ذي قار للفترة من كانون الثاني ولغاية ايار ٢٠٠٩ . جمعت عينات كيس الصفراء عشوائيا من ٧٥ فروج لحم في حاويات معقمة .
زرعت جميع العينات على كل من وسط أكار الدم ووسط أكار الماكونكي للتحري عن النمو البكتيري .
تم التعرف على العزلات النقية بأستخدام نظام الـ API 20E .
أوضحت هذه الدراسة أن أغلب العزلات البكتيرية المقاومة لمكونات الصفراء هي *Klesiella pneumonia* والتي عزلت من(٤٥) حالة نسبة ٦٠% ، بعدها جاءت *Proteus mirabilis* والتي عزلت من (٢٠) حالة بنسبة ٦و٢٦% ، بينما لم يتم الحصول على أي عزلة من النماذج المتبقية (١٠) والتي تشكل نسبة ٤و١٣%.