International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Isolation and Identification of Micro- Flora Streptococci from Chicken Intestine and the Effects of Antibiotic and Heavy Metals on Growth

Eman A. Mukhaifi[1], Salwa A. Abdul Jaleel[2]

Department of Biology, Faculty of Science, University of Basra, Iraq

Abstract: ***In present study we isolated Streptococcus bovis from the chicken intestine (duodenum, ileum, rectum) and fecal materials. The results founded all isolates can grow on nutrient agar, resistance to antibiotics Nitrofurantion(300 µg), ChloramphenicoI 30µg) Norfloxacin(10µg), Ampicillin (10µg) and diameter of inhibition (15mm) in Amikacin(10µg) compared to diameter of inhibition (mm) in control of Amikacin ≥ 17mm and, exhibited the highest MIC to certain heavy metals, MIC=14mM for pb^{+2} and MIC=18 mM for Cd^{+2}***

Keywords: streptococci, chicken intestine, heavy metal, antibiotic

1. Introduction

Chicken meat is the most eaten worldwide [1]. During chicken slaughtering, carcasses can be contaminated with fecal matters from the chicken's intestines. Bacterial infections due to *streptococcus sp.* have been contracted through such process or consumption of under cooked chicken meat [2, 4].

In practice, chickens are given antibiotics for either treatment or prophylaxis of infections, and for growth promotion to increase profits. These antibiotics belong to similar chemical categories to those used for treatment of microbial human infections. This raises concern on the possibility of human cross-infecting with chicken-infecting bacteria or the later transferring resistance traits to bacterial population that causes human infections [5, 9].

Chicks take place soon after hatching when the young animals ingest food. During the first 2 to 4 days, streptococci and enterobacteria colonize the small and large intestine.

Other studies on the development of the intestinal flora in chickens show qualitatively similar results in that lactobacilli and fecal streptococci can be isolated from the duodenum and ileum days old by using nonselective anaerobic rolltube culture media [7].

Previous studies have identified different types of bacteria in chicken meat and shown high antimicrobial resistance rates [11, 16]. The present study intended to isolate bacteria from chicken duodenum, ileum, rectum and fecal materials and assess antimicrobial resistance profiles with an antibiotic and heavy metals resistance.

2. Materials and Methods

Samples collection

Chickens sample were collected from different shops sells of chicken from different locations in Basra city., Chickens were slaughtered, dissected and the intestine were taken out and maintained in poly ethylene sack.

Isolation of intestinal bacteria

Approximately 5-cm lengths were taken from the duodenum, upper ileum and lower ileum, and 1g of feces were mixed with 100 ml distilled water into 250 ml flask and then put in a rotating shaker with a speed of 150 rpm at 30°C for 30 min from which 1ml transported from solution was pipette to 9ml distilled water. The purpose was to make 10^{-1} decimal and provide inocula of the dilutions usually 10^{-3} to 10^{-4} from the duodenum, 10^{-5} to 10^{-7} from the ileum, and feces, by sterilized pipette into plates containing nutrient agar[12].

Identification of bacterial isolates

Preliminarily, cells from isolate colonies stained by Gram stain. Cells appeared cocci slightly curved, white colonies, occurring small and tall chains and Gram-positive. After that inocula were transferred onto nutrient agar for purification, incubated for 24h and the isolates identified by morphological and biochemical tests as in Bergeys Manual of Determinative Bacteriology[8]. Isolates maintained on nutrient agar screw capped- tubes covered with 20% glycerol [10]. And identified by following tests: Gelatin hydrolysis, Starch Hydrolysis, nitrate reduction, Catalase, citrate utilization, salinity tolerance, motility, voges proskauer, Manitol salt ager and oxidation – fermentation [9].

Determination of antibiotic resistant

1ml inoculum of *Streptococcus bovis* was added to 100 ml of nutrient broth and then incubated at 30°C for 24h and then dilution of bacterial solution with Physiological Normal saline compared with the standard test tube McFarland for 108 cells / ml of stuck bacterial and inoculated into nutrient agar, using L-shap to spread bacteria on Muller Hinton media, and then but antibiotic disc on bacteria and incubated dishes in the incubator for 37 ºC. Then measured the diameter of the inhibition [13].

Determination of MIC of *Streptococcus bovis*

Streptococcus bovis isolates were inoculated into nutrient broth, supplemented with different heavy metal ions. The concentrations of heavy metal in the nutrient broth medium were:

$CdCl_2.H_2O$ 4, 6, 8, 10.12, 14, 16, 18, 20 mM
$(PbNO_3)_{2.}.4H_2O$ 4, 6, 8, 10, 12, 14, 16, 18, 20 mM[12].

3. Result

Isolates of *Streptococcus bovis* from chicken duodenum, ileum, rectum and fecal materials were twenty (20) isolates. All isolates can grow on nutrient agar. Identification was done according to Bergey's Manual of Determinative Bacteriology [8] (Figures 1, 2)(Table 1)

Figure 1: *S. bovis* grown on nutrient agar magnification 4.1X

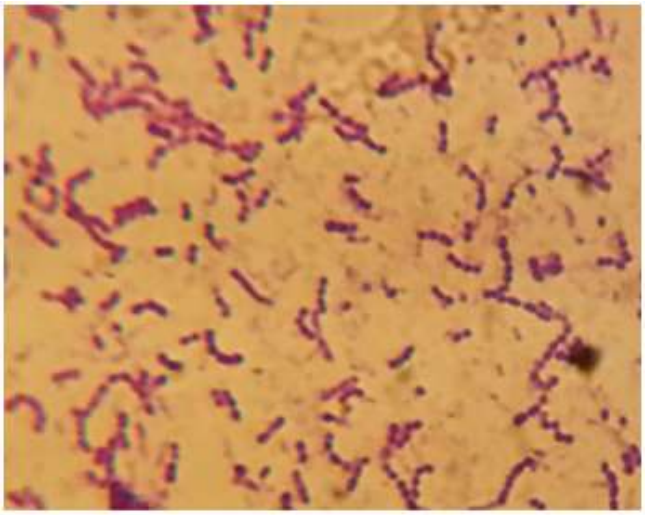

Figure 2: *S. bovis* with Gram stain magnification 1000X

Table 1: Results of morphological, physiological and biochemical tests of *S. bovis*

Characterization	*S. bovis*
Colony on M-BA(Methelen Blue Azide)	White small pinpoint
Catalase	-
NaCl 2%	+
NaCl 6.5%	-
Growth at 45C°	+
Growth at 10C°, 50C	-
lactose, fructose, glucose, raffinose and cellobiose	+
xylose, mannitol and glycerol	-
Blood hemolysis	-
Potassium tellurite 0.04%	-
Production of lactic acid	low
Production of NH3 from arginine	-
Hydrolysis of starch	+
Fermentation of Arabinose	-

The isolates which were selected from chicken duodenum, ileum, rectum and fecal materials resistance to antibiotics Nitrofurantion(300 µg), ChloramphenicoI 30µg) Norfloxacin(10µg), Ampicillin (10µg) and diameter of inhibition (15mm) in Amikacin(10µg) compared to diameter of inhibition (mm) in control of Amikacin ≥ 17mm (Figure 2).

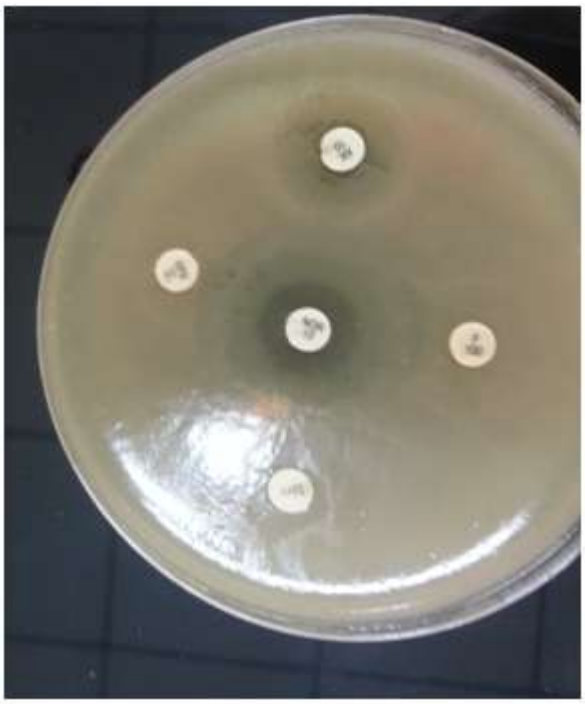

Figure 3: Antibiotics testes for *S.bovis*

F: Nitrofurantion, C:Chloramphenicol NOR:Norfloxacin and AK: Amikacin

The *S.bovis* which were selected from chicken duodenum, ileum, rectum and fecal materials resistance, exhibited the highest MIC to certain heavy metals, MIC=14mM for pb^{+2} and MIC=18 mM for Cd^{+2}, LSD=0.06800 with $p<0.01$.(Figure 4**).**

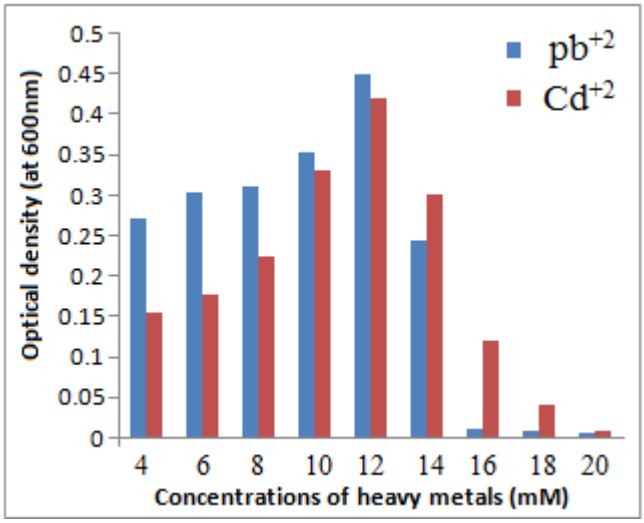

Figure 4: Minimum inhibitory concentrations (MICs) of *S.bovis* in mM of heavy metals

4. Discussion

From results in figure 1 the Spread plating is the way appropriate to isolate the bacteria from chicken duodenum, ileum, rectum and fecal materials. There are a number of procedures available for the isolation of micro organisms from mixed culture. But the initial and the most simpler method of isolation is spread plating on solid agar medium. The purpose of spreading is to isolate individual bacteria.

The majority of bacterial species that have been found chicken intestinal are those that are part of the normal flora of the tract and body, *S. bovis* is commonly found in the alimentary tract of chicken, cattle, sheep, and other ruminants [3] and may cause ruminal acidosis or feedlot bloat. It is also associated with spontaneous bacterial peritonitis, a frequent complication occurring in patients affected by cirrhosis. he main portal of entry for human infection of *S. bovis* bacteremia is the gastrointestinal tract, but in some cases, entry is through the urinary tract, the hepatobiliary tree, or the oropharynx [6].

From result in figure 2 Amikacin is effects on most bacteria combared to control due to most of bacteria produce a cell wall that is composed partly of a macromolecule called peptidoglycan, itself made up of amino sugars and short peptides. Amikacin prevents the final cross-linking step, or transpeptidation, in assembly of this macromolecule and inhibits bacterial growth by stopping protein synthesis. Both bacteria and humans carry out protein synthesis on structures called ribosomes. Norfloxacin can cross the membranes of bacteria and accumulate in high concentrations in the cytoplasm. Amikacin then binds to a single site on the ribosome--the 30S (smaller) ribosomal subunit--and blocks a key RNA interaction, which shuts off the lengthening protein chain [16].

Resistance due to most of bacteria The three fundamental mechanisms of antimicrobial resistance are (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics. Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome. The most important mechanism of resistance to the Amikicin and Ampicillin is antibiotic hydrolysis mediated by the bacterial enzyme beta-lactamase. Resistance to antibiotic, which is stable to gram-positive beta-lactamase, occurs through the alteration of an antibiotic

target protein, Ampicillin -binding protein 2. Production of antibiotic-modifying enzymes and synthesis of antibiotic-insensitive bacterial targets are the primary resistance mechanisms for the other classes of antibiotics, including Nitrofuration Noroflaxin and chloramphenicol [15].

The MIC value for pb and Cd in this study (14 mM) and 18(mM) respectively (figure 4) is higher than that obtained by Owolabi and Hekeu [14] which ranged between2 to 4 mM. These studies suggest variability in the potency of bacteria towards heavy metals to which they are resistant. This might be due to the fact that the metal resistance trait in *S.bovis.* is regulated by genes which are organized in operons. Generally, since the regulation of the metal resistant genes expression is specific for each heavy metal and is dependent upon metal species.

Resistance to Pb^{+2} is regulated by pbr operon, Pb^{+2} ions and enter the cell through transporters for essential metals. Upon the presence of intracellular metals, transcription of the *pbr* operon is initiated and consequently PbrA and PbrB proteins are produced. PbrA starts to pump and leads to the cell wall, while PbrB dephosphorylates substrates yielding inorganic phosphate. Free Pb^{+2} concentration decreases as Pb^{+2} is sequestered as a phosphate salt. The sequestration of lead discontinues the expression of the *pbr* operon [17].The resistant to Cd^{+2} by expression of the *CadA* and *CadC* located in cell wall.These proteins are encoded by cad operon. Upon the presence of intracellular metals, transcription of the cad operon is initiated and consequently *CadA* and *CadC* proteins are produced. *CadA* starts to pump cadmium to the cell wall and required ATP for cadmium transport, while *CadC* gene is known to be coding for binding protein of cadmium [18]. The minimal inhibitory concentration (MIC) of these heavy metal ions is a function of the complex dissociation constants of the respective sulfides by binding to SH groups, the heavy metal ions may inhibit the activity or the functioning of sensitive enzymes. The MIC is defined as the minimum concentration of a heavy metal at which microbial growth is completely inhibited by toxicity of heavy metal ion [19].

References

[1] KJ. Murphy, B. Parker, KA. Dyer, CR. Davis, AM. Coates, JD.Buckley, *et a*,. "A comparison of regular consumption of fresh lean pork, beef and chicken on body composition: a randomized cross-over trial" Nutrients, vol.6, pp.682-96, 2014.

[2] GB.Havenstein, PR. Ferket, MA. Qureshi" Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets". Poultry Science, vol.82, pp.1509-18, 2003.

[3] PJ. Panisello, R.Rooney, PC.Quantick, R.Stanwell-Smith, " Application of food borne disease outbreak data in the development and maintenance of HACCP systems." International journal of Food Microbiology, vol.59, pp.221–34, 2000.

[4] S.Omulo, SM.Thumbi, MK, Njenga, DR. Call, " A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better" Antimicrobial Resistance and Infection Control, vol., 4:1, 2015.

[5] GG.Khachatourians, " Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria", CMAJ.JAMC.vol.159, no.9, pp.1129-1136.1998.

[6] R.Horner, "Spontaneous bacterial peritonitis caused by *Streptococcus bovis*: case report and review of the literature, ". Scielo. Brazilian Journal of. InfectionDisese, vol.4no.14, 2015.

[7] E.M.Barnes, G. C. Mead, D. A. Barnum, and G. C.Harry, " The intestinal flora of the chicken in the period 2 to 6 weeks of age with particular reference to the anaerobic bacteria, "Br. Poul. Sci. vol, 13, pp. 311-326, 1972.

[8] B.W.William, D.V. Paul, M.G. George, J.Dorothy, R.K. Noel, L. Wolfgang, A.R. Fred H.H.and Karl, " Bergey's Manual of Systematic Bacteriology" Second Edition Vol.3, 2009.

[9] S.T. Cowan, J.G. Holt, J.Liston, R.G.E.Murry, C.F.Niven, A.W.Ravin and R.Y.Stanier "Bergy' s Manual of Determination Bacteriology ".8thED. Baltimore, USA.33-46.1974.

[10] I.LiterakM., Dolejska, D.Janoszowska, J.Hrusakova, W. Meissner, H.Rzyska, *et al, "* Antibiotic-resistant*Escherichia coli* bacteria, including strains with genes encoding the extended-spectrum beta-lactamase and QnrS, in waterbirds on the Baltic sea coast of Poland, " Appl Environ Microbiology, vol. 76, pp.8126–34, 2010

[11] JM.Miranda, AC. Mondraón, B.Martinez, M.Guarddon, JA.Rodriguez, " Prevalence and antimicrobial resistance patterns of Salmonella from different raw foods in Mexico, " J Food Prot, vol. 72, pp.966, 2009.

[12] Jp. Salanitro, I.G. Blake, P.A. Muirhead, M. Maglio, and R. Andj, "Bacteria Isolated from the Duodenum, Ileum, " App. And Enviro. Micro. Vol. 35, no.(4): 782-790, 1978.

[13] D.M.W.Kennedy,. and S.S. Wilbard,, ".Antimicrobial resistance profiles of bacteria isolated from chicken dropping in Dar Es Salaam, " Intern. J. of Pharm.and Pharmaceutical Sci, vol.7, no. 9, 0975-1491, 2015.

[14] J.B. Owolabi,. And M.M. Hekeu, "Heavy Metal Resistance and Antibiotic Susceptibility Pattern of Bacteria Isolated from Selected Polluted Soils in Lagos and Ota, Nigeria, " International Journal. of Basic & Applied Science. vol.14, no.06, pp. 142206-7373, 2014.

[15] L. Garcia-Migura, RS.Hendriksen, L.Fraile, FM.Aarestrup, " Antimicrobial resistance of zoonotic and commensal bacteria in Europe: the missing link between consumption and resistance in veterinary medicine, " Vet Microbiol, vol. 170, no.1-9, 2014.

[16] B.Jean, R. Franklin, A. Patricia, M.George, A. Janet, G. Stephen, S. James and D. Pharm, "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement, vol. 35 no. 3, 2015.

[17] T.M.Roane, I.L. and Pepper, "Microorganisms and metal pollution, " Environmental Microbiology, vol.3 no. 55, 2000.

[18] D.H. Nies, " Microbial heavy metal resistance, " Appl. Micr. Biotechnol. Vol. 51, pp.730-750, 1999.

[19] D.H.Nies, " Efflux-mediated heavy metal resistance in prokaryotes, "FEMS Microbiology Review, vol. 27, pp.313-39, 2003