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Short communication

The *in-vitro* Antibiotic Sensitivity Test of *Pasteurella multocida* Isolated from Layer and Breeder Chickens

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ABSTRACT

The current study aimed to characterize *Pasteurella multocida* (*P. multocida*) isolates from layers and breeder chickens in Egypt regarding *in-vitro* antibiotic sensitivity and resistance pattern. In doing so, spleen, liver, lungs, and heart, were taken aseptically from chickens suffering from a drop in egg production, septicemia, respiratory manifestations, and mortalities between 2016 and 2017. To isolate bacteria, samples were grown on a modified Das medium. Moreover, microscopic appearance and biochemical characteristics were used to identify pure colonies of *P. multocida* isolates. In the next step, in-vitro antibiotic sensitivity testing was performed on the isolated P. multocida. The findings indicated that *P. multocida* was found in 36 isolates out of 330 investigated chicken flocks. Small glistering, mucoid, grayish, and dew drop *P. multocida* colonies were discovered during the culture analysis. *Pasteurella multocida* isolates were Gram-negative coccobacilli using the microscope. Catalase, indole generation, H2S production, nitrate reduction, and oxidase tests were all positive for the sample; however, methyl red, urease activity, Voge's proskaur, and gelatin liquefaction tests were all negative. They also fermented glucose, mannose, fructose, sucrose, mannitol, xylose, and sorbitol without producing gas but not lactose, arabinose, maltose, inositol, salicin, raffinose, or dulcitol. Isolated *P. multocida* strains were sensitive to tetracycline, erythromycin, trimethoprim/sulphamethoxazole, norfloxacin, ofloxacin, penicillin, chloramphenicol, and azithromycin, while resistant to ampicillin and clindamycin. Cefoperazone, gentamycin, and streptomycin all showed intermediate sensitivity.

1. Introduction

Fowl cholera (FC) is a contagious disease caused by Gram-negative bacteria, *Pasteurella multocida* (*P. multocida*). This disease remains a significant obstacle for poultry production in many countries in the world as it causes severe economic losses for domestic and backyard birds[1,2]. Fowl cholera takes different infection forms, including peracute, acute with high mortalities and morbidities, and chronic localized ones[3]. The bacterium, *P. multocida*, is usually present in the upper respiratory tract, pharynx, and cloacae of birds. Thus, isolation and identification of the organism from clinical samples are very important for the diagnosis of the disease. Vaccines are used against FC, but the infection remains in poultry flocks.

Antimicrobials resistance of bacteria has become a great problem in human and veterinary medicine[4]. Different antimicrobials have been widely used for the treatment of *P. multocida* with varying results depending on the species, time, geographical origin, and the type of the used drug[5,6]. Strains of *P. multocida* are susceptible to most of the widely used commercial antimicrobial agents. However, haphazard, indiscreet, and prolonged usage of antimicrobials for the treatment of *P. multocida* accelerate the emergence of multidrug resistance to commonly used chemotherapeutic agents[7]. The antibiotic resistance increases the incidence of *P. multocida* infection and subsequently affects the economy of the locality.

Therefore, the aim of this study was to characterize *P. multocida* isolates from the Egyptian layer and breeder

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chicken flocks and determine the *in vitro* antibiotic sensitivity of isolates to different antimicrobial agents.

2. Materials and Methods

2.1. Bacteriology

Samples were collected from layers and breeder chicken flocks inEl-Sharqia, El-Gharbia, El-Qalubia, and El-Minofia governorates, Egypt during the period from 2016 to 2017. The flocks suffered from respiratory manifestations, septicemia, drop in egg production, and mortalities. The samples of liver, heart, spleen, and lungs were collected from freshly dead birds, inoculated in brain heart broth, and incubated at 37° C for 18-24 hrs. Subsequent selective sub-culturing of *P. multocida* isolates was done on modified Das media under aerobic conditions at 37°C for 48 hours to obtain pure cultures[8]. Gram staining was used for morphological identification of colonies[9]. Biochemical identification was made according to Quinn et al.[10].

2.2. In-vitro antibiotic sensitivity test

Isolated strains of *P. multocida* were tested for their susceptibility to 13 antimicrobial agents obtained from Oxoid Laboratories, UK The antibiotic discs were norfloxacin (NOR, 10 µg), gentamycin (CN, 10 µg), tetracycline (TE, 30 µg), erythromycin (E, 15 µg), streptomycin (S, 10 µg), cefoperazone (CEP, 75 µg), trimethoprim/sulphamethoxazole (SXT, 1.25/23.75 µg), ampicillin (AM, 10 µg), ofloxacin (OFX, 5 µg), chloramphenicol (C, 30 µg), penicillin G (P, 10 µg), azithromycin (AZM, 15 µg), and clindamycin (DA, 2 µg). Pure *P. multocida* colonies were picked and suspended in sterile saline and the turbidity was adjusted to 0.5 Mcfarland standard tube. The sterile cotton swab was dipped into the prepared inoculum tube, spread uniformly into Muller Hinton agar. The antibiotic discs were dispensed on the surface of the agar using forceps and the plates were incubated at 37°C for 24 hours. The zones of inhibition were measured and recorded to determine the sensitivity or resistance of *P. multocida* to the tested drug according to the standardized protocol by the Clinical and Laboratory Standards Institute[11].

3. Results and Discussion

Pasteurella multocida is the cause of avian cholera, a disease that has been described worldwide and causes great losses to the poultry industry[12]. Healthy carriers and chronic forms of the infection were well described[13]. Antimicrobial treatments have been extensively used for *P. multocida* with varying success[5].

Isolation of *P. multocida* on DAS media showed small glistering, grayish, mucoid, and dew drop colonies. Gram-negative coccobacilli were observed in stained smears from suspected *P. multocida* colonies. Suspected *P. multocid*a isolates were positive for catalase, oxidase, indole production, nitrate reduction, and H_2S production tests, while negative for methyl red, Voges-Proskauer, urease activity, and gelatin liquefaction tests. Moreover, they fermented glucose, fructose, mannose, mannitol, sucrose, sorbitol, and xylose without gas production but not arabinose, inositol, lactose, maltose, salicin, dulcitol, and raffinose. These findings are in accordance with Kawamota[14], Arora et al.[7], Purushothaman et al.[15], and Balasubramanium and Gopalakrishnamurthy[16]. Isolation of *P. multocida* from the liver of chickens was recorded[17].

The sensitivity of *P. multocida* to different antibiotics is shown in Table (1) and Figure (1). In the present study, the result of *in-vitro* antibiotic sensitivity test indicated that *P. multocida* was sensitive to ofloxacin, tetracycline, trimethoprim/sulphamethoxazole, penicillin, chloramphenicol, norfloxacin, azithromycin, and erythromycin, while resistant to ampicillin and clindamycin. Intermediate sensitivity was observed for cefoperazone, gentamycin, and streptomycin.

Sarangi and Panda[18] studied the antibiotic sensitivity test of *P. multocida* isolates and found that the organisms were sensitive to enrofloxacin, gentamycin, levofloxacin, gatifloxacin, and chloramphenicol, but resistant to penicillin G, streptomycin, sulfadiazine, cephalexin, cephotaxim, and ampicillin.

Table 1. Results of *in-vitro* sensitivity test of *P. multocida* against different antimicrobial agents

Agent	Potency (µg)	Standard sensitivity zone (mm)			Zone of inhibition (mm)	Interpretation
		R	I	S		
Ofloxacin (OFX)	5	12	13-15	16	28	S
Cefoperazone (CEP)	75	15	16-20	21	28	I
Gentamycin (CN)	10	12	13-14	15	14	I
Tetracycline (TE)	30	11	12-14	15	24	S
Streptomycin (S)	10	11	12-14	15	14	I
Ampicillin (AM)	10	13	14-16	17	0	R
Trimethoprim/ sulphamethoxazole (SXT)	1.25/23.75	10	11-15	16	19	S
Penicillin G (P)	10	21	22-28	29	30	S
Chloramphenicol (C)	30	12	13-17	18	29	S
Clindamycin (DA)	2	14	15-16	17	0	R
Norfloxacin (NOR)	10	12	13-16	17	29	S
Azithromycin (AZM)	15	≤ 12		≥ 13	26	S
Erythromycin (E)	15	12	13-15	16	18	S

S: sensitive I: intermediate R: resistant

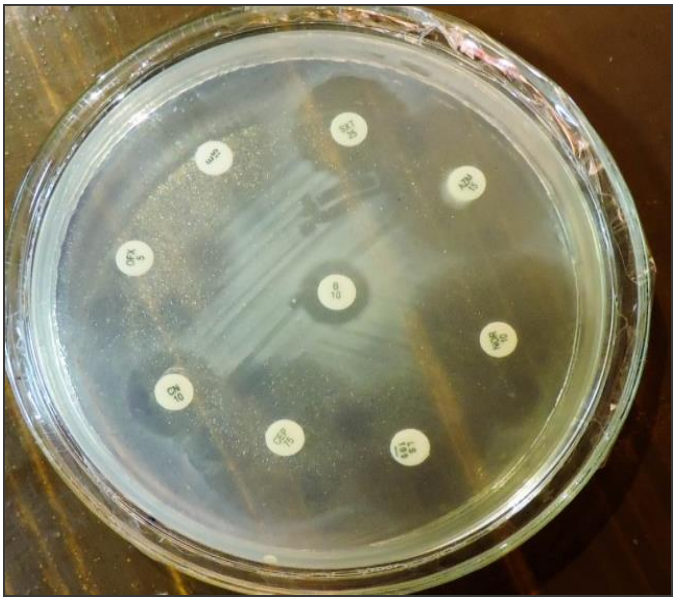

Figure 1. Results of antibiogram test of *Pasteurella multocida*

Similar sensitivity was recorded by Hirsh et al.[19] and Shivachandra et al.[20,] who demonstrated the susceptibility of *P. multocida* to chloramphenicol, enrofloxacin, gentamycin, tetracycline, penicillin G., streptomycin, sulphonamides, and trimethoprim. Moreover, Kamruzzaman et al.[21] recorded that *P. multocida* isolates in ducks were sensitive to ciprofloxacin and azithromycin, and showed intermediate sensitivity to gentamycin, tetracycline, amoxicillin, and erythromycin. Opposite results were obtained by Victor et al.[22,] who found the resistance of *P. multocida* to ofloxacin, ciprofloxacin, enrofloxacin, furasol, ceftazidime, and cefuroxime.

Strains of *P. multocida* vary in their susceptibility to different chemotherapeutics. Atere et al.[23] demonstrated that the multidrug resistance of *P. multocida* is attributed to the extensive application of antibiotics as additives in feed and extensive use of antimicrobial agents by poultry flocks. Antimicrobial resistance in *P. multocida* has been linked to small plasmids[24,25]. The coexistence and spread of these small plasmids resulted in multi-resistance of *P. multocida* isolates[26]. Moreover, this variation in the sensitivity pattern among different studies may be due to the excessive or limited previous exposure and/or indiscriminate use of antibiotics for prevention and control of infection[21]. In this study, the antimicrobial resistance was at a low level, which might be due to no resistance of the *P. multocida* isolates. The isolated *P. multocida* stains may not have previously or extensively been exposed to most of the tested antibiotics in the sensitivity test.

4. Conclusion

In this study, *P. multocida* was isolated and characterized biochemically from layer and breeder chicken flocks. The *in-vitro* antibiotic study revealed that *P. multocida* was sensitive to ofloxacin, tetracycline, trimethoprim/sulphamethoxazole, penicillin, chloramphenicol, norfloxacin, azithromycin, and erythromycin and these drugs could be successfully used for the treatment. It is recommended to use an antibiogram study before the treatment of *P. multocida* infection to select the most effective antibiotics.

Declarations

Competing interest

The authors declare that they have no competing interests.

Author's contribution

Ali Z. Qandoos collected samples, characterized the organism, and perform the antibiotic sensitivity test, Hanan A. Ahmed helped and supervised the laboratory work. Wafaa A. Abd El-Ghany supervised the experiment, wrote the draft of the manuscript, and check the final version of the manuscript.

Availability of data and materials

All collected data and related studies are done for publishing in the present journal.

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Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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