***In- vitro* antibiotic susceptibility pattern of avian *Pasteurella multocida***

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**Abstract**

This work was designed to characterize *Pasteurella multocida* (*P. multocida*) isolates from layers and breeders chickens flocks in Egypt with emphasis on *in-vitro* antibiotic sensitivity and resistant pattern. Liver, heart, spleen and lungs were collected aseptically from diseased birds suffered from respiratory manifestations, septicaemia, drop in egg production and mortalities during the period from 2016-2017. Samples were cultured on modified Das media for isolation of bacteria. Pure colonies of *P. multocida* isolates were identified according to microscopic morphology and biochemical characters. The isolated *P. multocida* were subjected to *in-vitro* antibiotic sensitivity test. Cultural study revealed small glistering, grayish, mucoid and dew drop *P. multocida* colonies. Microscopically, *P. multocida* isolates were Gram negative coccobacill. All the isolates were positive for catalase, oxidase, indol production, nitrate reduction and H2S production tests, while negative for methyl red, Voge’s proskaur, urease activity and gelatin liquefaction tests. Moreover, they fermented glucose, fructose, mannose, mannitol, sucrose, sorbitol and xylose without gas production but not ferment arabinose, inositol, lactose, maltose, salicin, dulcitol and raffinose. Isolated *P. multocida* isolates 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.

**Key words:** *Pasteurella multocida*, Antimicrobials, chickens, Egypt.

**Introduction**

Fowl cholera (FC) is a contagious disease caused by Gram negative bacteria, *Pasteurella multocida* (*P. multocida*). FC remains significant obstacle for poultry production in many countries in the world as it causes severe economic losses for domestic and backyard birds (Office International Dez Epizootics, 2008 and Moemen *et al.*, 2012). FC takes different infection forms vary from peracute and acute with high mortalities and morbidities as well as chronic localized ones (Christensen and Bisgaard, 2000). *P. multocida* is present in the upper respiratory tract, pharynx and cloacae of birds, so, isolation and identification of the organism from clinical samples is very important for the diagnosis of the disease. Though, vaccines are used against FC, but the infection still remains in poultry flocks.

Antimicrobials resistance of bacteria has become a great problem in human and Veterinary Medicine (Levy, 1998). Therapy using antimicrobials has been used widely for the treatment of *P. multocida* with varying results depending on species, time, geographical origin and the kind of drug used (Rimler and Glisson, 1997 and Caprioli *et al.*, 2000). Strains of *P. multocida* are susceptible to most of the widely used commercial antimicrobial agents. However, haphazard, indiscreet and prolonged use of antimicrobials for treatment of *P. multocida* accelerates the emergence of multidrug resistance to commonly used chemotherapeutic agents (Arora *et al.*, 2005). The antibiotic resistance increases the incidence of *P. multocida* infection and subsequently affects the economy of the locality.

So, the aim of this work was to characterize Egyptian *P. multocida* isolates as well as determine the *in vitro* antibiotic sensitivity of *P. multocida* to different antimicrobial agents.

**Materials ad Methods**

**Bacteriology**

Samples were collected from layers and breeders chickens flocks of different Egyptian governorates during the period from 2016-2017. Flocks were suffered from respiratory manifestations, septicaemia, drop in egg production and mortalities. Liver, heart, spleen and lungs were taken from freshly dead birds and inoculated in brain heart broth and incubated at 37° C for 18-24 hrs. Subsequent selective subculture *P. multocida* isolates was done on modified Das media under aerobic conditions at 37°C for 28 hrs to obtain pure cultures (Cowan, 1985). Colonies were stained with Gram’s for morphological identification (Kumar *et al*., 2004). Biochemical identification was made as Quinn *et al*. (1994).

***In-vitro* antibiotic sensitivity test:**

Isolated strains of *P. multocida* were tested for their susceptibility to 13 antimicrobial agents obtained from Oxoid Laboratories. 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. Sterile cotton swab was dipped into the prepared inoculum tube and spread uniformly into Muller Hinton agar, then antibiotic discs were dispensed on the surface of the agar using forceps and the plates were incubated at 37oC for 24 hr. The zones of inhibition were measured and recorded to determine the sensitivity or resistance of *P. multocida* to tested drug according to the standardized protocol by the Clinical and Laboratory Standards Institute (CLSI, 2017).

**Results and Discussion**

*P. multocida* is the cause of avian cholera, a disease that has been described worldwide and causes great losses to the poultry industry (Pedersen *et al.*, 2003). Healthy carriers and chronic forms of the infection are well described (Muhairwa *et al.*, 2000). Antimicrobial treatment has been used extensively used for *P. multocida* with varying success (Rimler and Glisson, 1997).

Isolation of *P. multocida* on DAS media showed small glistering, grayish, mucoid and dew drop. Gram negative coccobacilli were observed in stained smears from suspected *P. multocida* colonies. Suspected *P. multocid*a isolates were positive for catalase, oxidase, indol production, nitrate reduction and H2S production tests, while negative for methyl red, Voge’s proskaur, urease activity and gelatin liquefaction tests. Moreover, they fermented glucose, fructose, mannose, mannitol, sucrose, sorbitol and xylose without gas production but not ferment arabinose, inositol, lactose, maltose, salicin, dulcitol and raffinose. These findings are in accordance with Kawamota, (1990), OIE (2004), Arora *et al.* (2005), Purushothaman *et al.* (2008) and Balasubramanium and Gopalakrishnamurthy (2009). Isolation of *P. multocida* from liver of chicken was recorded by Dashe *et al.* (2013).

The susceptibility of *P. multocida* to different antibiotics is listed in Table (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 (2011) studied the antibiotic sensitivity test of *P. multocida* isolats and found that the organisms were sensitive to enrofloxacin, gentamycin, levofloxacin, gatifloxacin, chloramphenicol and resistant to penicillin G, streptomycin, sulfadiazine, cephalexin, cephotaxim and ampicillin.

Similar sensitivity were recorded by Hirsh *et al.* (1989) and Shivachandra *et al.* (2004) who found susceptibility of *P. multocida* to chloramphenicol, enrofloxacin, gentamycin, tetracycline, penicillin G., streptomycin and sulphonamide and trimethoprim. Moreover, Kamruzzaman *et al.* (2016) recorded that *P. multocida* isolates in ducks were sensitive to ciprofloxacin and azithromycin, intermediate sensitive to gentamycin, tetracycline, amoxicillin and erythromycin. Opposite results were obtained by Victor *et al.* (2016) who found resistance of *P. multocida* to ofloxacin, ciprofloxacin, enrofloxacin, furasol, ceftazidime and cefuroxime.

Strains of *P. multocida* vary to their susceptibility to different chemotherapeutics

Atere *et al.* (2015) demonstrated that the multidrug resistance of *P. multocida* is attributed to multi use 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 (Rosenau *et al.*, 1991 and Everlon *et al.*, 2013). The coexistence and spread of these small plasmids has resulted in multi-resistant of *P. multocida* isolates (San Millan *et al.*, 2009).

Variation in the sensitivity patterns among different studies may be due to over or limited previous exposure and/or indiscriminate use of antibiotics for prevention and control (Kamruzzaman *et al.*, 2016). In this work, the antimicrobial resistance was low which might be due to the isolated *P. multocida* don’t not acquiring resistance or undergoing selection pressure.

**Conclusion**

In conclusion, in this study *P. multocida* was isolated and characterized biochemically from layers and breeders chickens flocks. The *in-vitro* antibiotic study revealed that *P. multocida* was sensitive to ofloxacin, tetracycline, trimethoprim/sulphamethoxazole, penicillin, chloramphenicol, norfloxacin, azithromycin, and erythromycin that could be used for *P. multocida* treatment.

It is recommended using of antibiogram study before treatment of *P. multocida* infection to select the most effective drug.

Table (1): Results of *in-vitro* sensitivity test of *P. multocida* against different 13 antimicrobial agents

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| --- | --- | --- | --- | --- |
| **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

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