Original Research Article

Phenotypic and genotypic characterization of oxidase positive Gram negative bacilli isolated from broiler chickens.

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ABSTRACT

The current work aimed to study the phenotypic and genotypic characters of oxidase positive Gram negative bacterial pathogens recovered from different pathological lesions in broiler chickens. Samples were taken from 200 Hubbard and Ross broiler chickens of different ages (3-5weeks), from different farms in Beni-Suef and El-Fayoum Governorates during the period from January 2016 to April 2016. Bacteriological examination showed that Gram negative bacteria were 165 (82.5%) of isolates of which 60 isolates (30%) were oxidase negative while 105 isolates (52.5%) were oxidase positive including 43 *Pseudomonas aeruginosa*, 35 *Aeromonas hydrophila*, 12 *Pasteurella gallicida*, 10 *Plesiomonas shigelloides*, and 5 *Vibrio vulnificus* with incidences of 21.5%, 17.5%, 6% 5%, and 2.5%, respectively. The *in-vitro* sensitivity tests were applied on a total of 59 isolates; 20 *P. aeruginosa*, 19 *A. hydrophila*, 10 *P. gallicida*, 5 *P. shigelloides* and 5 *V. vulnificus* against 13 different antimicrobial agents and multidrug resistant isolates were detected. Multiplex-PCR was applied on 15 different MDR isolates. The results of PCR revealed that bla_{TEM}, *CIT* and *FOX* genes were the most prevalent where they were found in 8 isolates (53.3%) followed by bla_{SHV} which was found only in 5 isolates (33.3%).

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1. Introduction

Miscellaneous Gram negative bacteria are seriously affecting broiler chickens and poultry industry in Egypt. *P aeruginosa* is the most predominant Pseudomonas species causing infection, mortality among birds and clinical signs including septicemia, diarrhea and respiratory signs (**El-Shafii, 1992 and Tanios and Kamel, 1999**). Also, members of the genus *Pasteurellacae*

are the causative agent of numerous, economically important diseases, including avian fowl cholera and other disorders (**De Alwis, 1992**). Fowl cholera is a serious disease of poultry and can present in either acute or chronic forms. Obvious clinical signs of acute fowl cholera may not occur until very late in the infection and include depression, ruffled feathers, fever, anorexia, mucous discharge from the mouth, diarrhoea and an increased respiratory rate (**Rhoades and Rimler, 1989**).

Moreover, Aeromonas infections in poultry have been reported in different parts of the world with devastating effects (**Dashe *et al*., 2013**). A higher occurrence of Aeromonas from chicken source suggests that chicken could be a potential host for the spread of Aeromonas infection and present a possible threat to public health due to the ubiquitous nature of Aeromonas in aquatic, clinical and environmental sources (**Smita and Brahmabhatt, 2011**). Also, *Plesiomonas shigelloides* (previously *Aeromonas shigelloides*) are ubiquitous, facultative anaerobic, flagellated, Gram-negative rods (**San Joaquin, 1994**). *P. shigelloides* have been isolated from a variety of environmental sources (**Jeppesen, 1995**).

Furthermore, the genus Vibrio includes several food-borne pathogens that cause a spectrum of clinical conditions including septicemia, cholera and milder forms of gastroenteritis. Several *Vibrio* species are commonly associated with food-borne transmission including *V. cholerae*, *V. parahemolyticus*, and *V. vulnificus* (**Azwai *et al*., 2016**).

Although antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive birds, antimicrobial drug use has been implicated as a risk factor in the development and dissemination of drug resistance (**Gosh and LaPara, 2007; Radwan *et al*., 2016**). Food of animal origin and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact or indirectly via the food production chain (**WHO, 2011**). Therefore, the appropriate antibiotic should better

be selected on the basis of its sensitivity which could be detected by laboratory examination. The recovery of antimicrobial-resistant isolates in foods of animal origin has raised concerns that the treatment may be compromised because antimicrobial-resistant strains appear to be more often associated with severe disease than are susceptible isolates

The aim of this study was to study the phenotypic and genotypic characters of oxidase positive Gram negative bacterial pathogens recovered from different pathological lesions in broiler chickens.

2. Materials and Methods

2.1. Samples

Samples were taken from 200 Hubbard and Ross broiler chickens of different ages (3-5 weeks), from different farms in Beni-Suef and El-Fayoum Governorates during the period from January 2016 to April 2016.

These chickens were suffered from respiratory manifestations (coughing, sneezing, ralling, nasal discharge and sometimes swelling of infra orbital sinuses either bilateral or unilateral).The chickens were subjected to clinical, postmortem and bacteriological examination of the affected tissues including liver (*n=95*), kidney (*n=50*), pericardium (*n=29*) and air sacs (*n= 26*).

2.2 Bacteriological examination

The collected samples were cultivated under aseptic condition into Tryptone Soya broth. All inoculated media were incubated aerobically at 37C for 24 hrs. Then loopful from the inoculated broth were streaked onto MacConkey's agar, tryptone soya agar (TSA) and dextrose starch agar. The colonies were examined for their cultural characters and morphological appearance according to **Mahon *et al*. (2015)**.

Medium sized colonies from MacConkey agar and dextrose starch agar were picked up for purification on TSA and incubated aerobically at 37C for 24-72 hrs. Smears from separate colonies and from livers of suspected fowl cholera cases were stained with Gram's and Leishman's stains and examined microscopically. Colonies revealed

pure Gram negative bacilli, showing bipolarity were inoculated onto nutrient slopes.

For each plate, one single colony representing typical colonial appearance and morphological character was picked up and inoculated into 12% Glycerol broth then kept at -20C for further investigation.

All the recovered Gram negative, medium size and non sporulated isolates (with Gram's stain smears) were further examined biochemically.

2.3. Biochemical identification of the obtained bacterial isolates.

2.3.1 By using conventional biochemical tests.

The following tests were adapted for identification of bacterial isolates: oxidase, TSI, indole production, methyl red (MR),Voges Prauskeur (VP), citrate utilization, hydrogen sulphide (H_2S) production on TSI, urease activity, nitrate reduction, gelatin liquefaction and sugar fermentation for glucose, lactose, sucrose, mannose, arabinose, maltose and mannitol according to (Collee *et al*. 1996). Other tests such as haemolysis onto blood agar (β), motility, growth at 4C, Growth at 42C and pigment production were included.

2.3.2. Identification by using API kit

The appropriate API kit (API20NE, Oxoid) was used according to the manufacturer's instruction.

2.3.3. Microbact-24E bacterial identification system

Is a commercial microsystem simplify the identification of Enterobacteriaceae and common miscellaneous Gram-negative bacilli, including oxidase positive GNBs, consists of dehydrated substrates distributed in the wells of microtitre trays with the Thermo Scientific™ Oxoid™ Microbact™ GNB that Kit is a complete, self-contained biochemical based identification system. It uses 24 different biochemical tests in microplate format to produce easy-to-read, distinct color reactions,

generally following overnight incubation. Interpret using the Thermo Scientific™ Microbact™ Identification Package) and this was done according to the manufacturer's instruction.

2.4. Antimicrobial susceptibility testing

A total of 59 isolates; 20 *P. aeruginosa*, 19 *A. hydrophila*, 10 *P. gallicida*, 5 *P. shigelloides* and 5 *V. vulnificus* were selected and tested for their antimicrobial susceptibility to 13 different antimicrobial discs including ampicillin (10μg), gentamycin (30μg), amikacin (30μg), ciprofloxacin (5μg), sulphamethoxazole-trimethoprim (25μg),levofloxacin (5μg), doxycycline (30μg), aztreonam (30μg), kanamycin (30μg), apramycin (15μg), colistin sulphate (10μg), erythromycin (15μg) and amoxicillin clavulanic acid (30μg) (Oxoid Limited, Basing Stoke, UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to CLSI (2015). The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the CLSI (2015). Resistance to three/or more antimicrobials of different classes was taken as multidrug resistant (MDR) (Chandran *et al*., 2008).

1.1. Multiplex-PCR for detection of β-lactamases resistance and virulence genes

DNA was extracted by using bacterial DNA extraction kits (Qiagen, Germany, GmbH) according to the manufacturer instructions. The multiplex-PCR assay was applied on 15 different MDR isolates (4 *P. aeruginosa,* 4 *A. hydrophila,* 3 *P. gallicida,* 2 *P. shigelloides* and 2 *V. vulnificus*) for detection of 4 resistance genes (*bla$_{TEM}$*, *CIT*, *bla$_{SHV}$* and *FOX*). Targeted genes and their primer sequences are listed in table (1).

3. Results

Table (1). Primer sequences and amplified products for the resistance genes.

Genes		Primer Sequence5'-3'	Amplified product	Reference
bla_{TEM}	F	ATCAGCAATAAACCAGC		
	R	CCCCGAAGAACGTTTTC	516 bp	
bla_{SHV}	F	AGGATTGACTGCCTTTTTG		**Colom et al., 2003**
	R	ATTTGCTGATTTCGCTCG	392 bp	
CIT	F	TGG CCA GAA CTG ACA GGC AAA		
	R	TTT CTC CTG AAC GTG GCT GGC	462 bp	**Pérez-Pérez and Hanson, 2002**
FOX	F	AAC ATG GGG TAT CAG GGA GAT G		
	R	CAA AGC GCG TAA CCG GAT TGG	190 bp	

3.1. Prevalence of bacterial isolation from the diseased chickens.

The results recorded in **table (2)** showed that out of 200 chickens (aged 3-5 weeks) the total number of recovered isolates were 193 (96.5%). Gram negative bacterial isolates were 165 (82.5%) of which 105 isolates (52.5%) were oxidase positive and 60 isolates (30%) were oxidase negative. Moreover, 28 isolates (14%) were Gram positive. On the other hand, 7 samples (3.5%) had no bacterial isolation (negative isolation).

Table (2): Prevalence of bacterial pathogens obtained from diseased chickens:

Total No. of samples	Positive isolation										Negative isolation	
	Gram negative				Gram positive		Total isolation					
	Oxidase positive		Oxidase negative		Total							
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
200	105	52.5	60	30	165	82.5	28	14	193	96.5	7	3.5

%: calculated according to the total number of samples.

3.2. Biochemical identification of oxidase positive Gram negative bacterial isolates.

Identification of oxidase positive Gram negative bacterial isolates using traditional biochemical tests as well as API 20NE and Microbact systems revealed that the isolates were identified as *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Pasteurella gallicida*, *Plesiomonas shigelloides* and *Vibrio vulnificus*.

3.3. Prevalence of oxidase positive Gram negative bacterial isolates recovered from diseased chickens.

From the total collected samples (*n=200*) the most prevalent bacterial isolates were *Pseudomonas aeruginosa* (43 isolates), *Aeromonas hydrophila* (35 isolates), *Pasteurella*

gallicida (12 isolates), *Plesiomonas shigelloides* (10 isolates), and *Vibrio vulnificus* (5 isolates) with

incidences of 21.5%, 17.5%, 6% 5%, and 2.5%, respectively **(Table 3)**.

Table (3): Prevalence of oxidase positive Gram negative bacterial isolates recovered from diseased chickens.

Bacterial isolates	No.	%
Pseudomonas aeruginosa	43	21.5
Aeromonas hydrophila	35	17.5
Pasteurella gallicida	12	6
Plesiomonas shigelloides	10	5
Vibrio vulnificus	5	2.5
Total	**105**	**52.5**

No.: Number of isolates.
%: was calculated according to the total number of the examined cases.

3.4. Antimicrobial susceptibility testing.

Results of *in-vitro* sensitivity tests illustrated in **table (4)** showed that *P. aeruginosa* isolates were completely resistant to erythromycin, amoxicillin clavulinic acid, doxycycline, ampicillin and

apramycin while they were highly resistant to amikacin (80%) and sulphamethaxzole-trimethoprim (70%). On the other hand, they were highly sensitive to kanamycin and ciprofloxacin (80% of each). MDR were detected in 13/20 isolates (65%). Meanwhile, *A. hydrophila* isolates were completely resistant to sulphamethoxazole-trimethoprim and erythromycin while they were highly resistant to kanamycin and ampicillin (89.5% of each). On the contrary, they were highly sensitive to amikacin (94.7%) and colistin sulphate (73.3%). MDR were detected in 12/19 isolates (63%). Moreover, *P. gallicida* isolates were completely resistant to sulphamethoxazole-trimethoprim, erythromycin and doxycycline hydrochloride while they were highly resistant to apramycin (80%) and both of aztreonam and kanamycin (70% of each). On the other hand, they were completely sensitive to amoxicillin clavulanic acid while they were highly sensitive to amikacin (90%). MDR were detected in 6/10 isolates (60 %).

   P. shigelloides isolates were completely resistant to sulphamethoxazole-trimethoprim, erythromycin and doxycycline hydrochloride while they were highly resistant to amoxicillin clavulanic acid (80%). On the contrary, they were completely susceptible to amikacin, ciprofloxacin, levofloxacin, aztreonam and gentamycin while they were highly sensitive to kanamycin (80%). MDR were detected in 2/5 isolates (40 %). while, *V. vulnificus* isolates were completely resistant to sulphamethoxazole-trimethoprim, levofloxacin, aztreonam, kanamycin and ampicillin. On the other hand, they were completely susceptible to amoxicillin clavulanic acid and amikacin while they were highly sensitive to erythromycin and gentamycin (80% of each). MDR were detected in 4/5 isolates (80 %).

3.5. Multiplex-PCR for detection of resistance genes.

   Multiplex-PCR was applied on 15 different MDR isolates (4 *P. aeruginosa*, 4 *A. hydrophila*, 3 *P. gallicida*, 2 *P. shigelloides* and 2 *V. vulnificus*) for detection of 4 resistance genes (*bla*$_{TEM}$, *CIT*, *bla*$_{SHV}$ and *FOX*). The results revealed that *bla*$_{TEM}$, *CIT* and *FOX* genes were the most prevalent where they were found in 8 isolates (53.3%) followed by *bla*$_{SHV}$ which was found only in 5 isolates (33.3%) **(Table 5 and Fig. 1)**.

Table (5): Prevalence of resistance-associated genes among the examined MDR isolates using multiplex-PCR.

Tested gene	No. of examined isolates	Positive		Negative	
		No.	%	No.	%
bla$_{TEM}$		8	53.3	7	46.7
CIT	15	8	53.3	7	46.7
bla$_{SHV}$		5	33.3	10	66.7
FOX		8	53.3	7	46.7

% was calculated according to Number (No.) of examined isolates.

   Concerning *P. aeruginosa* tested isolates (*n=4*), *CIT* and *FOX* genes were the most prevalent and found in all isolates (100%) followed by *bla*$_{SHV}$ gene which was found in 3 isolates (75%) and finally *bla*$_{TEM}$ gene found in 2 isolates only (50%). Meanwhile, in *A. hydrophila* tested isolates (*n=4*), *bla*$_{TEM}$, *CIT* and *FOX* genes were found in 2 isolates (50%) while *bla*$_{SHV}$ gene was not detected at any isolates. While, in case of *P. gallicida* isolates (*n=3*), *CIT* and *bla*$_{SHV}$ genes were the most prevalent and found in 2 isolates (66.7%) followed by *bla*$_{TEM}$ gene which was found only in 1 isolate (33.3%) while *FOX* gene was not detected at any isolates. Moreover, in *P. shigelloides* isolates (*n=2*), *bla*$_{TEM}$ gene was the only gene detected and found in all isolates (100%) while the other genes were not detected at any isolates. Also, in *V. vulnificus* isolates (*n=2*), *FOX* gene was the most prevalent and found in all isolates (100%) followed by *bla*$_{TEM}$ gene which was found only in 1 isolates (50%) while *CIT* and *bla*$_{SHV}$ genes were not detected **(Table 6 and Fig. 1)**.

Table (6): Resistance-associated genes distribution among the examined MDR isolates using multiplex-PCR.

Genes	*P. aeruginosa* (no=4*)		*A. hydrophila* (no=4*)		*P. gallicida* (no=3*)		*P. shigelloides* (no=2*)		*V. vulnificus* (no=2*)	
	No.	%	No.	%	No.	%	No.	%	No.	%
bla$_{TEM}$	2	50	2	50	1	33.3	2	100	1	50
CIT	4	100	2	50	2	66.7	-	-	-	-
bla$_{SHV}$	3	75	-	-	2	66.7	-	-	-	-
FOX	4	100	2	50	-	-	-	-	2	100

No.: Number of positive cases.
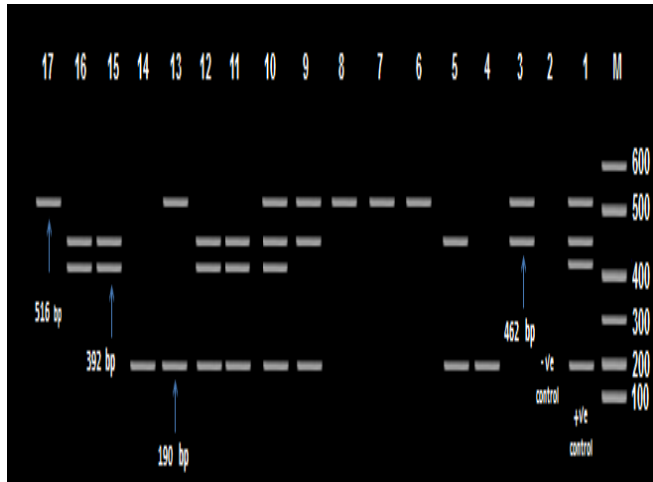**%: was calculated according to the corresponding number (no.) of examined isolates (*).**

Fig. (1):Multiplex-PCR amplification of the 516bp, 462bp, 392bp and 190bp fragments of *blaTEM, CIT, blaSHV* and *FOX* genes, respectively, from different isolates which showed a positive/negative amplicons migrates with the molecular DNA size marker (M).

- L1 (+ve control): positive control.
- L3-L6: *Aeromonas hydrophila* isolates.
- L9-L12: *Pseudomonas aeruginosa* isolates.
- L15-L17: *Pasteurella gallicida* isolates.

- L2 (-ve control): negative control.
- L7-L8: *Plesiomonas shigelloides* isolates.
  L13-L14: *Vibrio vulnificus* isolates.

4. Discussion

No doubt that bacterial agent causing significant economic losses in commercially produced poultry worldwide (**Barnes *et al*., 2003**).

The obtained results in table (2) are nearly coinciding with that reported by **Poornima and Upadhyde (1995) and Sedhom (2000)**. Samples with negative bacterial isolation could be attributed to viral, parasitic or fungal causatives (**De Herdt *et al*., 2008** and **Karki *et al.*, 2009**).

Results of traditional biochemical tests for bacterial identification are the same to the results obtained by **Sedhom (2000) and Emam (2006)**.

Concerning using MB24E and API20NE besides conventional biochemical methods for identification; the MB24E identifications were found to be "correct". Thus in their hands, the MB24E gave 100% correct identification, while the API20E was "correct" to species level in 97.4% of cases (**Mugg and Hill, 1981**).

It is common of poultry farmers in Egypt to administer antibiotic cocktails comprised of several human and veterinary preparations to birds, particularly when conventional poultry drugs fail to mitigate the clinical signs of disease (**Olarinmoye *et al*., 2013**). A disturbing but long-term consequence of this practice is the emergence and involvement of multidrug resistant phenotypes that are more difficult to treat in veterinary and human population with public health implications; this observation concurred with the reported by **Dashe *et al*. (2013)**. The need for *in-vitro* sensitivity test is limited in large animal practice but, it is very beneficial in poultry practice for prophylaxis programs. In poultry practice, thus sensitivity testing is intended to give a rational basis for the choice of an antimicrobial drug. The type of antibiotic used in the treatment should better be selected on the basis of antibiotic culture sensitivity test technique for treatment of respiratory affections in chickens (**Blanco *et al.*, 1997**).

In the current study, the *in-vitro* antimicrobial sensitivity tests were applied on a total of 59 didifferent bacterial isolates recovered from different pathological lesions including 20 *P. aeruginosa*, 19 *A. hydrophila*, 10 *P. gallicida*, 5 *P. shigelloides* and 5 *V. vulnificus*. These isolates were tested against 13 field used chemotherapeutic agents. The results shown in **Table (4)** revealed that *P. aeruginosa* isolates gave complete resistance against the most of antimicrobial used including erythromycin, amoxicillin clavulinic acid, doxycycline, ampicillin and apramycin while high resistances were recorded against amikacin and sulphamethaxzole trimethoprim. On the contrary, high sensitivity was recorded against kanamycin and ciprofloxacin and moderate sensitivity was recorded against levofloxacin (60%). These results were agreed with that recoded by **Hamed (1999) and Todar (2004)** who reported that *P. aeruginosa* were resistant to the most of antibiotics and only few antibiotics were effective against it. Also, these results were partially similar to **Abdel Gwad *et al*. (1998)** who recoded high sensitivity against garamycin, neomycin, danofloxacin, colistin and amikacin and moderately sensitive to chloramphenicol, lincomycin, nitrofurans, trimethoprim and cefotixin and **Hesham**

(2002) who found that the isolates were sensitive to amikacin, rifampicin and tetracycline.

The current study revealed that out of 20 *P. aeruginosa* isolates there were 13 isolates showing multidrug resistance (MDR) with incidence of 65%. **Aggarwal *et al.,* (2008)** investigated ESBL production in *P.aeruginosa*. They detected ESBL among 20.3% of the investigated isolates and all of them were multi-drug-resistant. Moreover, they recorded that carbapenems and ofloxacin were the most effective drugs with 100 and 70%.

P. aeruginosa is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane LPS. Also, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Moreover, *P. aeruginosa* maintains antibiotic resistance plasmids and it is able to transfer these genes by mean of the bacterial processes of transduction and conjugation. **(Todar, 2004).**

Moreover, *A. hydrophila* isolates gave complete resistance against both sulphamethoxazole trimethoprim and erythromycin while high resistances were recorded against kanamycin and ampicillin and moderately resistant to levofloxacin. On the contrary, high sensitivity was recorded against amikacin and colistin sulphate while moderate sensitivity was recorded against apramycin.

Presence of β-lactamase genes in Aeromonas has been reported by **Igbinosa and Okoh (2012)** which is an alarming public health concern. Member of the genus Aeromonas readily develop single or multiple antimicrobial resistance phenotypes and R-plasmids are commonly found, thus they were well suited for monitoring the prevalence of antibiotic resistance, as well as for investigating the conjugative spread of resistance genes **(Schmidt *et al.,* 2001)**. Since antibiotic resistance exists in bacteria in different and potentially linked reservoirs, an integrated laboratory-based surveillance programme for monitoring resistance in all relevant reservoirs is needed.

This study revealed that about 12 from 19 *A. hydrophila* isolates were multi drug resistance with an incidence of 63%. **Igbinosa *et al*.,(2012)** isolated a multi-drug resistant *A. hydrophila* from different parts of the world and are reported to be resistant to penicillin and ampicillin, but sensitive to aminoglycosides, tetracycline, chloramphenicol,

trimethoprim-sulfamethoxazole, quinolones, and second as well as third-generation cephalosporins also, a reported multi drug resistance of *A. hydrophila* isolated from chickens by. **(Kore *et al.*, 2014; Soltan *et al.*, 2015)**.

Additionely, *P. gallicida* isolates showed complete resistance against sulphamethoxazole trimethoprim, erythromycin and doxycycline hydrochloride and high resistances against apramycin, aztreonam and kanamycin in addition to moderate resistance to both of ampicillin and colistin sulphate (60% of each) which differ from **Huang *et al.* (2009)** found the resistance level to ampicillin and cephalotin to be less than 5%. **Shivachandra *et al.* (2004)** also reported much higher levels of resistance (ampicillin 23.58%; amikacin 55.28% and to tetracycline 24.39%) in study involving one hundred and twenty-three strains of *Pasteurella multocida* obtained from outbreaks of fowl cholera from different avian host and various geographical regions of India.

On the other hand, *P. gallicida* strains were completely sensitive to amoxicillin clavulanic acid while they were highly sensitive to amikacin (90%) and moderately sensitive to gentamicin, ciprofloxacin and levofloxacin were (60% of each). This was nearly supported by **Rahman *et al.,* (2004)** who reported complete sensitivity against ciprofloxacin and gentamicin and moderate sensitivity against ampicillin, cephradine and penicillin G while a complete resistance was recorded against tetracycline. Meanwhile, **Prabhakar *et al*., (2012)** recorded a complete sensitivity against ciprofloxacin and high sensitivity against gentamicin (93%) and enrofloxacin (90%). These results correlated with the current findings. Similar findings were also reported by **Sarangi and Panda (2011)** who reported a complete sensitivity against enrofloxacin and high sensitivity against gentamycin (85.7%), levofloxacin (85.7%), gatifloxacin (85.7%), chloramphenicol (71.4%) while high resistances were recorded against penicillin G (85.7%), streptomycin (85.7%), sulfadiazine (85.7%), cephalexin (71.4%), cephotaxim (71.4%) and ampicillin (71.4%). *The increased incidence of multidrug-resistant pathogenic bacteria has been widely reported in the last several decade.* **Huang *et al*.,(2009,** a multidrug-resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or

more antimicrobial categories by **Magiorakos *et al.,* (2012)**.

Also, *P. shigelloides* isolates were completely resistant to sulphamethoxazole trimethoprim, erythromycin and doxycycline hydrochloride. Meanwhile, they were highly resistant to amoxicillinclavulanic acid and moderately resistant to colistin sulphate, ampicillin and apramycin. Conversely, they were completely susceptible to amikacin, ciprofloxacin, levofloxacin, aztreonam and gentamycin while they were highly sensitive to kanamycin.

It was clear that P. shigelloides is naturally resistant to several antibiotics. Because the organism has the natural potential for resistance to a wide range of β-lactams under conceivable testing conditions it might be useful to describe *Plesiomonas* as naturally resistant to a variety of β-lactams, i.e. all penicillins but also some cephalosporins, like cefoperazone, ceftazidime and cefepime, and to renounce the use of these β-lactams in the treatment of severe *Plesiomonas* infections. **(Stock and Wiedemann, 2001)**.

Moreover, *V. vulnificus* isolates were completely resistant to sulphamethoxazole trimethoprim, levofloxacin, aztreonam, kanamycin and Ampicillin and moderately resistant to ciprofloxacin, colistin sulphate, doxycycline and apramycin. On the other hand, they were completely susceptible to amoxicillin clavulanic acid and amikacin while they were highly sensitive to erythromycin and gentamycin.

*Vibrio spp.*isolates were found to be resistant against most of the commonly used antibiotics in this study which may ensure the fact that antimicrobial resistance is increasing both in the farm animal and public health sectors and emerged as a global problem in recent decades due to improper selection, indiscriminate use and misuse of antimicrobials in food producing animal farms food and water Furthermore, contamination with antibiotic-resistant bacteria is a major threat to public health and therefore, the public health concern is increased over a concept that antibiotics fed to food producing animals may contribute to the resistance of human pathogens**(Teuber, 2001; Bywater, 2004)**.

The variation in the sensitivity grade among various studies may be due to over or limited previous exposure and/or indiscriminate use of antibiotics as feed additives and/or preventive or curative agents.

PCR technique is widely used in veterinary research and this technique is likely to have a strong impact in the epidemiology treatment and prevention of animal infections disease. however: laboratory procedures for isolation and identification of pathogens are laborious and time consuming, several virulence associated genes have been targeted for detection of potentially pathogenic **(Balakrishna *et al*.2010)**

MDR bacteria have been recognized as an increasing problem in the veterinary and medical fields, and mobile DNA elements, including plasmids, transposons, and integrons, facilitate the proliferation of resistance genes in bacteria using multiplex pcr **(Speer *et al*., 1992 and Liebert *et al*., 1999)** such as genes encoded the resistance to β-lactamase (e.g. *blaTEM*) Plasmids play an important role in virulence especially R-plasmids which have been extensively studied in view of the prevalence of MDR **(O'Brien et al., 1982)**.

5. Conclusion

The presence of drug resistance pathogens occurred due to the misuse of the antibiotics and it is considered a great problem. In this study, antimicrobial susceptibility testing of *P. aeruginosa*, *A. hydrophila*, *P. gallicida, p. shigelloides* and V. *vulnificu* was performed Furthermore, multidrug-resistant strains were characterized and Multiplex-PCR was applied on 15 different MDR isolates for virulence genes detection.

6. Acknowledgement

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