LIST OF CONTENTS

LIST OF ABBREVIATIONS

bp	Base pair
FAO	Food and Agriculture Organization
*fli*C	Flagellin gene
*hil*A	Hyper invasive locus A gene
*inv*A	Invasion gene
LPS	Lipopolysaccharide
MDR	Multidrug resistance
MR	Methyl red
*pef*A	Plasmid encoded fimbriae Agene
PCR	Polymerase chain reaction
QRT-PCR	Quantitative real time- Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
r.p.m	Round per minute
RRP	Response regulator protein
RT-PCR	Reverse trancriptase polymerase chain reaction
RV	Rappaport - Vassiliadis
*sef*C	Salmonella enteritidis fimbrial C gene
sip	Salmonella invasion protein gene
*sop*B	Salmonella outer protein B gene
SPI	Salmonella pathogenicity island
SPP	Species
spv	Salmonella virulence plasmid gene
stn	Salmonella enterotoxin gene
TCI	T-cell inhibitior protein
TSI	Triple sugar iron
TTSS	Type three secretion system
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholate agar

SUMMARY

Poultry is one of the most important reservoirs of Salmonellae that can be transmitted to humans through the food-chain causing high risk of bacterial food poisoning.

Pathogenesis of Salmonella depends on a large number of factors controlled by an array of genes responsible for the actual virulence of Salmonella. Furthermore, multidrug resistance of Salmonella to commonly used antimicrobials is increasing both in the veterinary and public health sectors and has emerged as a global problem that lead to treatment failure.

Therefore, the outcome of this work was to assess the value of using PCR in rapid detection of virulence genes among multidrug resistant (MDR) Salmonellae which is of utmost importance to establish effective infection control measures in Sharkia Governorate.

In the present work, bacteriological examination of 300 samples from broiler internal organs (liver, spleen and heart) with a previous history of diarrhea revealed 30 salmonella isolates (10%)

Conventional methods for isolation and identification of Salmonella isolates from chickens showed that Salmonella appeared as colorless colonies on MacConkey's agar medium. It gave the characteristic slightly transparent zone of reddish color with or without black center on XLD agar medium and colorless colonies with black center on SS agar medium. It appeared as Gram negative, straight rods, non spore forming and arranged singly, in pairs and in groups. Moreover, Salmonella isolates were citrate test positive (blue

color), urease test negative (yellow color) and they also gave acid butt (yellow) and alkaline slant (red) with H_2S production (black coloration) on TSI agar medium.

Results of API 20 E for identification of 25 of Salmonella isolates from different sources were in parallel to the conventional biochemical identification results and revealed different 7 digit profile numbers.

Serotyping of 30 different Salmonella isolates by slide agglutination test using specific monovalent and polyvalent O and H Salmonella sera revealed seven different Salmonella serogroups, with *Salmonella* Typhimurium as the most prevalent serotype (46.7%), followed by *Salmonella* Enteritidis (20%), *S.* Kentucky and *S.* Arizona (10% for each one) while *S.* Montevideo, *S.* Birkenhead , *S.* Virchow were the least detected serovars isolate (6.6, 3.3 and 3.3%, respectively).

Antimicrobial sensitivity test revealed that all Salmonella isolates were sensitive to ciprofloxacin and chloramphenicol indicating their efficiency as the drugs of choice against Salmonella infections, while they were all resistant to erythromycin, amoxicillin/clavulanic acid and rifamycin. *Salmonella* Enteritidis was the most sensitive serotype.

Additionally, all isolates were resistant to at least 3antibiotics and multidrug resistance was mostly observed in *S.* Birkenhead and *S.*Typhimurium serotypes as *S.* Birkenhead was resistant to 8 antibiotics and 2 isolates of *S.* Typhimurium were resistant to 7 antibiotics.

According to variations in the diameters of inhibition zones of antimicrobial agents against Salmonella of the same serotypes,

Salmonella Typhimurium and *Salmonella* Enteritidis were actually differentiated except 2 strains of S. Typhimurium that could not be differentiated according to their inhibition zone diameters, but they were differentiated genotypically.

Regarding PCR detection of 8 virulence genes of 17 MDR salmonella isolates which proved to play an important role in the virulence of Salmonella in chicken. The results showed that PCR based methods with genus-specific primers belonging to *inv*A gene, is a reliable technique for confirmation of Salmonella isolates as all 17 *Salmonella* species which identified phenotypically were found to posses *inv*A gene (100%).

Species identification of most common Salmonella serotype as *S*. Typhimurium and *S*. Enteritidis was performed using specific genes as *fli*C and *sef*C, respectively. The result revealed that all *S*.Typhimurium were positive for amplification of 620 bp fragments of *fli*C gene and only *S*. Enteritidis isolates gave positive amplification of 1103 bp fragments of *sef*C gene.

In addition, *hil*A gene was detected in 88.2% of different Salmonella isolates and produced a PCR product at 854 bp.

Furthermore, it was proved that *pef*A, *stn* and *sop*B virulence genes were detected in most Salmonella serotypes by percentages of 41.1%, 58.8% and 41.1%, with the production of characteristic bands at 700, 617, 1348 bp, respectively.

On the other hand, the virulence plasmid of Salmonella (*spv*C) was presented only in *Salmonella* Enteritidis and gave a characteristic band at 669 bp indicating its specificity to *S*. Enteritidis.

The *hil*A was the most predominant gene, followed by *stn* gene being present in 15 and 9 Salmonella isolates with percentages of 88.2% and 58.8%, respectively. PCR results could also differentiate the two *S*. Typhimurium isolates that could not be differentiated phenotypically by disc diffusion test.

It was revealed that multidrug resistant Salmonella serotypes were also capable for exhibiting several virulence determinants which are very important to induce Salmonella pathogenicity. Not only the most common Salmonella serotypes incriminated in chickens outbreaks, *S.*Typhimurium and *S.* Enteritidis, but also there were other multidrug resistant and virulent Salmonella serotypes (*S.*Birkenhead, *S.* Virchow *S.* Kentucky, *S.* Arizona and *S.*Montevideo).

Finally, the association between phenotypic antimicrobial results and genotypic detection of some virulence genes of different *Salmonella* species could be effective in providing a more accurate profile for understanding the dangerous spread of virulence genotypes and antibiotic resistance in *Salmonella* species.

Despite the large of virulence genes that have been found in different Salmonella serovars, *S.* Enteritidis and *S.* Typhimuriun were up to 90% identical at the genetic level and share many important phenotypic characteristic, thus it is assumed that both pathogens have many different mechanisms in common.

Multidrug resistant Salmonella serotypes were also capable for exhibiting several determinants at least one gene.

CONCLUSION

The outcome of this work was to assess the value of using PCR in rapid detection of the most important virulence genes among the multidrug resistant (MDR) different Salmonella serotypes isolated from broilers at different localities in Sharkia Governorate.

This study could be concluded in the following points:

- The incidence rate of Salmonella was a relatively low (10%).
- Salmonella Typhimurium and *S.* Enteritidis were the most prevalent ones followed by other species as, *S.* Kentucky, *S.*Arizona *S.* Montevideo, *S.* Birkenhead and *S.* Virchow.
- All Salmonella serotypes were sensitive to ciprofloxacin and chloramphenicol, being the drug of choice against Salmonella infection, while they were resistant to erythromycin, amoxicillin/clavulanic acid and rifamycin, being the least effective antimicrobial agents against *Salmonella* species.
- *Salmonella* Typhimurium was the most multidrug resistant serotype followed by *S.* Birkenhead, but *S.* Enteritidis was the most sensitive one.
- Uniplex PCR assay succeeded to detect 8 virulence genes (*inv*A, *fli*C, *sef*C, *hil*A, *sop*B, *stn*, *pef*A and *spv*C genes)
- All salmonella isolates identified phenotypically were found to possess *inv*A gene (100%), being *Salmonella* species specific gene thus confirming its affiliation to *Salmonella* species.
- Species identification of most common Salmonella serotype (*S.*Typhimurium and *S.* Enteritidis) could be performed by specific genes as *fli*C and *sef*C, respectively.

- The *hil*A gene was the most prevalent one among different Salmonella isolates irrespective of their serotype, making more confidence that the detection of this gene can be used for rapid diagnosis of Salmonella infection in poultry.
- *Pef*A, *stn* and *sop*B virulence genes were detected in most Salmonella serotypes indicating its pathogenicity and virulent strains.
- The virulence plasmid of Salmonella (*spv*C) was found only in *Salmonella* Enteritidis indicating its specificity to *S.* Enteritidis serotype.
- Multidrug resistant Salmonella serotypes were also capable for exhibiting several virulence determinants which are very important to induce Salmonella pathogenicity.
- Not only the most common Salmonella serotypes incriminated in chickens outbreaks, *S.* Typhimurium and *S.* Enteritidis, but also there were other multidrug resistant and virulent Salmonella serotypes (*S.* Birkenhead, *S.* Virchow *S.* Kentucky, *S.* Arizona and *S.* Montevideo).
- PCR results could differentiate the two *S.* Typhimurium isolates that could not be differentiated phenotypically by disc diffusion test.
- Association between phenotypic antimicrobial results and genotypic detection of some virulence genes could be effective in providing a more accurate profile for understanding the dangerous spread of virulence genotypes and antibiotic resistance in *Salmonella* species, being MDR salmonella contain at least one virulence gene.