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High Prevalence of Multi-drug Resistant Bacteria in Selected Poultry Farms in Selangor, Malaysia

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ABSTRACT

Prevalence of multidrug resistant bacteria in apparently healthy chickens from 3 selected poultry farms in Selangor area of Malaysia was investigated. Conventional isolation techniques such as growth on selective media, gram staining and biochemical tests were utilised for the identification of the different bacterial isolates. Antimicrobial sensitivity test was monitored with the disc diffusion assay against 12 antimicrobial agents. A total of 96 *Staphylococcus aureus*, 48 *E. coli*, 7 *Pasteurella* sp. and 6 *Salmonella* sp. were isolated. All *E. coli* and *Salmonella* spp. isolates were multidrug resistant while 77.2% of *Staphylococcus aureus* and 71.5% of *Pasteurella* sp. isolates were multidrug resistant. The study further revealed highest resistance to tetracycline while cephalothin as the best drug of choice for treatment of infections caused by the isolates in the study area. Since not only chickens are at risk, this study recommends urgent intervention by regulatory agencies to limit the emergence and spread of these bacteria as well as prudent use of antibacterial agents among farmers in Malaysia.

Key words: Multidrug, resistant, bacteria, chickens, Malaysia

INTRODUCTION

The introduction of antimicrobial agents in the early 20th Century is one of the greatest achievements of scientific medicine. However, the use of these antimicrobial agents for clinical purposes and growth promotion purposes (Gunal *et al*., 2006) has increased the number of organisms that developed resistance to these agents. Previous studies showed that for many antimicrobial agents, resistant bacteria were found within three to five years from the introduction of the antimicrobial agent into clinical use (Schwarz and Chaslus-Dancla, 2001; Swartz, 2002). Antibiotic resistance still remains a global problem today (Adeleke and Omafuvbe, 2011).

Serious infections, notably in hospitals and other health care facilities are associated with the emergence of antibiotic-resistant organisms (Schwartz *et al*., 1997; Spellberg *et al*., 2008; Taddele *et al*., 2012). The treatment of infections caused by antibiotic-resistant organisms is difficult because of limited options and the organisms appear to be biologically competent to cause serious threat to life (Mulvey and Simor, 2009; Sibi *et al*., 2011). The continued discovery of these large

number of drug-resistant organisms is occurring at a time of decreased discovery and development of new anti-infective agents (Menghani *et al*., 2011; Mamun-or-Rashid *et al*., 2012) and most of the new agents are synthetic relatives of the older ones (Spellberg *et al*., 2008). As a consequence, an increase in the number of infections which cannot be treated is imminent in the near future.

Although most of the antimicrobial resistance problems in human medicine stem from overuse, there is evidence that antimicrobial resistant enteric bacteria can transfer from animals to humans and thereby establishing a reservoir of resistant genes (Fey *et al*., 2000; Angulo *et al*., 2004; Molbak, 2004; Maripandi and Al-Salamah, 2010). The widespread use of antibiotics in animals has also raised several concerns related to human and animal health. The principal area of concern has been the increasing emergence of antibiotic resistance phenotypes in both clinically relevant strains and normal commensal microbiota (Chikwendu *et al*., 2008). The use of nontherapeutic levels of antibiotics in poultry production can select for antibiotic resistance in commensal and pathogenic bacteria in poultry (O'Brien, 2002). Transfer of resistant bacteria between animals and humans through food products has been documented and can pose a threat to public health (O'Brien, 2002; Angulo *et al*., 2004; Molbak, 2004; Sornplang *et al*., 2011; Akinjogunla *et al*., 2011).

The World Health Organisation (WHO) has recognised that antimicrobial resistance is a global problem that calls for a global response. Consequently, WHO issued the global principles for the containment of antimicrobial resistance in animals intended for food and the WHO global strategy for the containment of antimicrobial resistance where some interventions were recommended that hopefully will enable local authorities to slow down the emergence and reduce the spread of resistance in diverse settings (WHO, 2000, 2001). These guidelines recommend the establishment of surveillance programmes for antimicrobial consumption and resistance, as well as guidelines for prudent use of antimicrobials and further research.

Antimicrobial resistance rates of bacteria from animals and their products are available for many countries (Centers for Disease Control and Prevention, 2004; Bywater *et al*., 2004; SWARM, 2007; Getachew *et al*., 2010). However, continuous monitoring is important for the control of resistance in animals and man (WHO, 2000, 2001). Continuous monitoring and surveillance will efficiently evaluate the resistance problem and detect trends and changes (WHO, 2000, 2001). Monitoring will identify the prevalence of resistance and resistance trends over time can determine the emergence of resistance, help to develop guidelines for the prudent use of antimicrobials and limit the emergence and spread of resistant organisms (WHO, 2000, 2001).

Multi-drug resistant bacteria have earlier been reported in other animal species in Malaysia (Zunita *et al*., 2008; Ooi *et al*., 2011). The present study is aimed at determining the prevalence of multi-drug resistant bacteria from selected poultry farms in Selangor area, Malaysia.

MATERIALS AND METHODS

Sampling: Three commercial poultry farms were selected from Selangor area of Malaysia where a total of 80 samples from apparently healthy chickens were collected comprising of skin and feather swabs for *Staphylococcus aureus*, nasal and tracheal swabs for *pasteurella* and cloacal swabs for *E. coli* and *Salmonella*. All samples were collected in January 2011 using sterile swabs.

Isolation and identification of bacterial isolates: Conventional isolation techniques such as growth on selective media, gram staining and biochemical tests were utilised for the identification of the different bacterial isolates. The *Staphylococcus aureus* isolates from both skin and feather were identified and confirmed based on colony and cell morphology, gram positive staining, positive

catalase and coagulase tests and formation of yellow colonies on Mannitol salt agar. *E. coli* isolates were identified and confirmed using colony and cell morphology, pinkish colonies on MacConkey agar, gram negative staining, indole and methyl red positive, Voges-Proskauer and citrate negative. *Salmonella* isolates were identified and confirmed using colony and cell morphology, pinkish colonies on brilliant green agar and positive for motility test. The *Pasteurella* isolated were identified and confirmed based on colony and cell morphology on blood agar, gram negative staining and oxidase positive test.

Antimicrobial sensitivity test: Antimicrobial sensitivity test was monitored with the disc diffusion assay (Kirby-Bauer) recommended by the NCCLS (2000) and CLSI (2010) on Muller Hinton agar (Oxoid, Milan, Italy); with the following 12 antimicrobial agents : Ampicillin 10 μg (AMP 10), Ciprofloxacin 5 μg (CIP 5), Sulphamethoxazole/Trimethoprim 25 μg (SXT 25), Streptomycin 10 μg (S 10), Tetracycline 10 μg (TE 10), Cephalothin 30 μg (KF 30), Erythromycin 15 μg (E 15), Chloramphenicol 30 μg (C 30), Penicillin G 10 units (P 10), Oxacilin 1 μg (OX 1), Clindamycin 2 μg (DA 2) and Neomycin 30 μg (N 30) also obtained from Oxiod (Milan, Italy). Antibiotic agents were selected based on the importance in treatment of the bacterial isolates. The zone of inhibition was interpreted according to National Laboratory Standard Institute (NLSI, 2010). Although multidrug resistance is defined as resistance to ≥3 antibiotics tested (Oteo *et al*., 2005), resistance to ≥4 antibiotics tested was considered in this study.

RESULTS

A total of 96 *Staphylococcus aureus*, 48 *E. coli*, 7 *Pasteurella* spp. and 6 *Salmonella* spp. were isolated from the 3 poultry farms (Table 1). *Staphylococcus aureus* and *E. coli* were isolated from all the skin and feather and cloacal swabs respectively from all the farms. However, out of the 7 *Pasteurella* spp. isolated 4 were from tracheal specimens of farm A and 3 from nasal specimens of farm B. Neither the tracheal nor the nasal specimens from farm C yielded positive colonies for *Pasteurella*. The only 6 *Salmonella* spp. isolated were from cloacal specimens of farm B, none was isolated from farm A and C (Table 1).

The result of the antibiotic sensitivity tests for the bacterial isolates is presented in Table 2. More than 50% of the *Staphylococcus aureus* isolates showed resistance to ampicillin, tetracycline, erythromycin, penicillin and clindamycin, where as none of the isolates were resistance to cephalothin. Ninety four percent of *E. coli* isolates were resistant to tetracycline while on the hand only 33% of the isolates were resistant to cephalothin. All *Salmonella* isolated were found to be resistant to tetracycline and clindamycin while only 14% were resistant to cephalothin. Although no breakpoint for zone of inhibition was given for *Pasteurella,* isolates that have no zone of inhibition were considered as resistant. All the bacterial isolates in this study showed very high resistance (83-100%) to tetracycline while all isolates except *Salmonella* showed low resistance (0-33%) to cephalothin (Table 2).

Table 3 presents the resistance of the bacterial isolates to the number of antibiotics used in the study. Highest numbers of *Staphylococcus aureus* isolates (17.7%) were resistant to four antibiotic agents while on the other hand only 3.1% of the isolates were resistant to 10 antibiotic agents. A total of 77.2% of *Staphylococcus aureus* isolates were resistant to 4 or more antibiotic agents. For *E. coli* highest number of isolates (25%) was resistant to 9 different antibiotic agents while on the other hand lowest number of isolates (4.2%) was resistant to 5 and 12 antibiotic agents. All *E. coli*

Table 1: Source of samples and number of bacteria isolated from selected poultry farms in Selangor, Malaysia

		No. of samples					No. of organisms isolated			
Source	No. of birds	Skin	Feather	Nostril	Trachea	Cloaca	*Staph. aureus*	*E. coli*	*Pasteurella*	*Salmonella*
Farm A	6	6	6	6	6	12	36	18	4*	0
Farm B	5	5	5	5	5	10	30	15	3**	6
Farm C	5	5	5	5	5	10	30	15	0	0
Total	11	16	16	16	16	32	96	48	7	6

*: Tracheal sample, **: Nasal sample

Table 2: Antibiotic resistance of bacteria isolated from selected poultry farms in Selangor, Malaysia

	No. of antibiotic resistance isolates (%)											
	AMP 10	SXT 25	TE 10	E 15	P 10	DA 2	CIP 5	S 10	KF 30	C 30	OX 1	N 30
Bacterial isolates	(µg)											
Staph. aureus (n = 96)	51 (53)	13 (14)	82 (85)	94 (98)	51 (53)	86 (90)	39 (41)	NA	0 (0)	51 (53)	27 (28)	NA
E. coli (n = 48)	31 (65)	38 (79)	45 (94)	NA	NA	NA	24 (50)	32 (67)	16 (33)	37 (77)	NA	NA
Salmonella (n = 6)	6 (100)	1 (17)	5 (83)	NA	NA	NA	5 (83)	2 (33)	6 (100)	1 (17)	NA	NA
Pasteurella (n = 7)	NA	5 (71)	7 100)	2 (29)	5 (71)	7 (100)	5 (71)	5 (71)	1 (14)	5 (71)	5 (71)	3 (43)

NA: Not applicable in the 2010 guidelines

Table 3: Resistance of bacterial isolates to number of antibacterial agents tested

	Resistance of bacterial isolates to number of antibiotic agents (%)											
Bacterial isolates	1	2	3	4	5	6	7	8	9	10	11	12
Staph. aureus (n = 96)	0 (0)	6 (6.3)	16 (16.7)	17 (17.7)	11 (11.5)	16 (16.7)	7 (7.3)	11 (11.5)	9 (9.4)	3 (3.1)	0 (0)	0 (0)
E. coli (n = 48)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.2)	6 (12.5)	2 (4.2)	4 (8.3)	12 (25)	10 (20.8)	10 (20.8)	2 (4.2)
Pasteurella (n = 7)	0 (0)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	0 (0)	2 (28.6)	2 (28.6)	1 (14.3)	0 (0)	0 (0)
Salmonella (n = 6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.3)	1 (16.7)	2 (33.3)	0 (0)	0 (0)	1 (16.7)	0 (0)

isolates (100%) were resistant to 5 or more antibiotic agents. *Pasteurella* isolates presented 71.5% resistance to 8 and above antibiotic agents where as 100% of *Salmonella* isolates were resistant to 6 or more antibiotic agents.

DISCUSSION

The microbial isolates identified in this study include *Staphylococcus aureus, E. coli, Pasteurella* sp. and *Salmonella* sp. *Staphylococcus aureus* and *E. coli* appeared to be the most prevalent bacterial species isolated. *Staphylococcus aureus* is known to be easily carried in the nasopharynx, throat, skin, cuts, boils, nails and as such can easily contribute to the normal microflora (Ekhaise *et al*., 2008). *Salmonella* is the least bacteria isolated from the chickens and this trend can be attributed to the good *Salmonella* control programme practiced by most farms as examination of food to detect *Salmonella* is routinely carried out for food safety and food-borne disease surveillance.

The result of this study revealed the presence multidrug resistant bacteria from chickens. All isolates showed high resistance to tetracycline while on the other hand all except *Salmonella* spp. showed very low resistance to cephalothin. The result of this study clearly identified cephalothin as a good choice antibiotic for treatment of infection in the study area. Also all *E. coli* and *Salmonella* sp. isolated in this study were found to be resistant to 5 or more antibacterial agents tested in this study, a finding which is supported by earlier reports of Overdevest *et al*. (2011) that

drug resistance in Enterobacteriaceae has increased dramatically during the past decade. The increase which was attributed mainly to increased prevalence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (Canton *et al*., 2008; Coque *et al*., 2008; Hashim *et al*., 2011) has caused increase in the use of last-resort antimicrobial drugs (i.e., carbapenems).

In addition, these results provide evidence that there is an increased emergence of antibiotic resistance from commensal bacterial isolates, a finding which is in agreement with the earlier reports of Chikwendu *et al*. (2008) who found increasing emergence of antibiotic resistance phenotypes in both clinically relevant strains and normal commensal microbiota. The presence of multidrug-resistant bacteria significantly limits the treatment options available for these life-threatening infections. This unfortunate trend is happening at a time when the discovery and development of new antibacterial agents has slowed down drastically as reported by Spellberg *et al*. (2008) and Menghani *et al*. (2011).

Although the control of antibiotic usage in Malaysia is legislated, the findings of this study indicated a probable indiscriminate use of antibiotics by the poultry farms in Selangor. This is further supported by earlier reports of uncontrolled used of antibiotics in feed and for treatment by some selected pig farms in Malaysia by Ooi *et al*. (2011). Although the use of antibiotics in human medicine has influenced the emergence of antibiotic-resistant bacteria, the use of antibiotics in animal agriculture has markedly contributed to this problem as indicated in this study. This has raised several concerns related to human and animal health. The types of bacteria detected from the poultry farms investigated in this study are associated with a variety of human infections (Gehanno *et al*., 2009; Lim *et al*., 2009; Fadel and Ismail, 2009). Especially the fact that transfer of resistant bacteria between animals and humans through food products has been documented and can pose a threat to public health (O'Brien, 2002; Angulo *et al*., 2004; Molbak, 2004). Although antimicrobial resistance rates of bacteria from animals and their products are available for many countries (Bywater *et al*., 2004; SWARM, 2007) including Malaysia (Getachew *et al*., 2010), the guidelines issued by WHO recommends continues surveillance and prudent use of antibacterial agents. The findings in this investigation emphasize the importance of studying multiple genera of bacteria from different animals as sources of human exposure to antibiotic resistance strains. Therefore not only that the chickens are at risks, poultry workers and consumers are equally exposed to serious hazards due to multidrug resistance bacteria. This calls for urgent intervention by regulatory agencies to limit the emergence and spread of these bacteria as well as prudent use of antibacterial agents among farmers in Malaysia.

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