

Multidrug Resistant-*Proteus Mirabilis* Isolated from Chicken Droppings in Commercial Poultry Farms: Bio-security Concern and Emerging Public Health Threat in Bangladesh

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Abstract

Poultry is a rampantly expanding agro-industry in Bangladesh like other developing countries. Several studies have detected multidrug resistance (MDR) *Proteus mirabilis* from poultry meat globally; however, no similar data was available for poultry samples in Bangladesh. *P. mirabilis* is a zoonotic human pathogen of urinary tract infection (UTI), nosocomial infection and wound infection, therefore, a potential threat to public health. We isolated *P. mirabilis* from chicken droppings collected from local commercial poultry farms and examined their antimicrobials susceptibilities. Chicken droppings were streak-cultured onto xylose lysine deoxycholate agar plates after enriching in buffered peptone water. Selective colonies were identified by biochemical test and API20E kits. Antimicrobial susceptibilities were tested by Kirby-Bauer disk diffusion method. Total 36 *P. mirabilis* were isolated from 39% (27 of 70) chicken droppings. Tetracycline evidenced as the highest individually-resistant (94%, 34/36) antibiotic (AB) while ciprofloxacin was the lowest (17%, 6/36). Hazard lies when 83% *P. mirabilis* were proved to be MDR (30/36), being resistant to three or more AB. Findings provide a baseline data on MDR *P. mirabilis* circulating around these PFs, it would assist the veterinarian in rational treatment and biosafety planning. More detail studies will be required to clarify their antimicrobial resistance and clinical relevance.

Keywords: *Proteus mirabilis*; Antimicrobial resistance; Poultry; Chicken droppings; Bangladesh

Introduction

Poultry remains the largest domestic animal stock in the world in terms of the number of animals [1]. The industry has expanded extensively in commercial levels as well as in household traditional levels in Bangladesh. More than three million peoples are employed directly in poultry sector, which provides the largest supply of meat and eggs [2], so as to meet up the major protein sources for entire population of the country. Since raising small-scale commercial poultry farms (PFs) demands low investment, it has been expanding at a high rate, mostly at the rural and semi-urban areas which contribute in national economic growth, considerably. These PFs are often run by unskilled, non-professional managers having poor knowledge on biosecurity alike other developing countries [3]. Since most of these PFs neither do have a good surveillance systems nor well-documented monitoring mechanisms to record potential pathogenic microorganisms or other poultry-hazards claiming serious public health implications. Recent data from various poultry based studies in Bangladesh evidences high prevalence of human pathogens like, *Escherichia coli*, non-typhoidal *Salmonella enterica* and *Enterobacter spp* [2,4,5], being similar to other countries reporting various *enterobacteriaceae* in eggs and meats [6,7]. Several authors reported presence of *P. mirabilis* in poultry meat [8,9]; none of similar type of data had been reported from in Bangladesh. Thus the aforementioned facts prompted us to investigate the presence of *P. mirabilis* in chicken droppings of Bangladeshi poultry that might be transmitting this zoonotic pathogen [10] to vulnerable workers while handling infected chicken directly or through fecal-contaminated poultry products as similarly *enterobacteriaceae* have been reported to transmit [11,12].

In Bangladesh, wide-spectrum antibiotics are often used irrationally (misused/overused) due to lack of adequate education and shortfall in mass awareness among most of the work-forces. Poor surveillance,

less quality assurance parameters, lacks in monitoring and gaps in regulatory mechanisms has thrown the overall public health situation in greater threat [2]. Such malpractices in antimicrobial uses have been reported to exert selection pressure of antimicrobial resistance to gut-microbial flora in poultry [13,14]; including recent evidences of multi drug resistance (MDR) *P. mirabilis* [9]. This *P. mirabilis* is known human pathogen as a common cause of human urinary tract infection (UTI), nosocomial infection, wound infection [15] and showed clear history of zoonosis in wide host ranges with emergence of MDR in recent years [10,16]. MDR *P. mirabilis* may therefore be transmitted among PF-workers who in turn may transmit that in surrounding environment thus infecting the catchment population at large. Therefore, we wanted to measure susceptibilities/resistance of the detected *P. mirabilis* to selected antimicrobials (AB) in our limited settings.

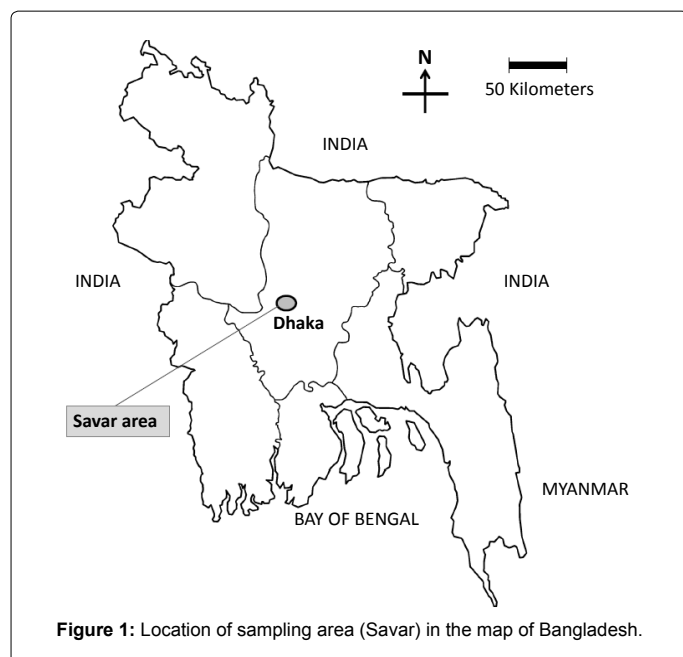
We conducted this cross-sectional survey to dig out a baseline data to see if it serves the purpose of linking up further public health research to determine if *P. mirabilis* have plausible zoonotic association in causing human diseases like UTIs, particularly among PF-workers and its catchment population. We anticipate these findings would assist the veterinarians and clinicians in planning more rational antibacterial therapy and biosafety policy in adopting stringent

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Poultry Farm*	Sample(s)	No. (%) of positive Samples	No. of isolates
F1	CK001-010	0 (0)	0
F2	CK011-020	0 (0)	0
F3	CK021-030	7 (70)	8
F4	CK031-040	3 (30)	5
F5	CK041-050	4 (40)	5
F6	CK051-060	4 (40)	7
F7	CK061-070	9 (90)	11
Total	CK071-080	27 (38.6)	36

*Ten samples of chicken droppings were collected from each of the seven different farms located in sub-urban area of Savar, Bangladesh.

Table 1: prevalence of *P. mirabilis* in chicken droppings.

surveillance and regular monitoring in these PFs thus augmenting the disease prevention and control strategies against poultry based *Proteus*-infection, effectively.

Methods

Study area description and sample collection

More than half of the PFs in Bangladesh are situated at the periphery of Dhaka among the 64 districts of the country [17]. Savar, a sub-district, located about 30 kilometer north-west of Dhaka city (Figure 1), is an industrial area. The vast majority of industries including small-, medium- and large- scale poultry and related agro-farms have been established in this region. Seven small-scale poultry farms were randomly selected from 4 distinct semi/peri-urban areas of Savar (*Khas Mahal, South Rajason, Pan Dhoya and Islamnagar*) for sampling of chick-dropping during a four months period (Apr-Jul, 2012). Three of those farms were cultivating broiler type chickens, three with layer-chickens and one with pre-starter broiler chickens. We collected 70 different samples, 10 from each farm, aseptically, when a unique code number for each farm and an identity-number for respective sample was assigned carefully (Table 1).

Sample preparation

Following utmost precautions chicken droppings were collected so as to prevent probable cross contaminations. Samples were immediately

stored in pre-labeled plastic containers in insulated ice-boxes and were transported to Jahangirnagar University laboratory within an hour where all microbiological examination were carried out. One loopful from each chicken dropping was diluted aseptically within 200µl sterile PBS in Eppendorf tubes and mixed properly using vortex mixture. Diluted samples were inoculated on specific culture media and the left-out samples were safely stored overnight at 4°C safely to repeat microbiological analyses, if required.

Culture procedures and identification techniques

We modified the traditional *Salmonella* isolation protocol to isolate *Proteus spp.* Diluted poultry droppings were enriched in buffered peptone water, (HIMEDIA, India) and one loopful of broth was streaked onto xylose lysine deoxycholate (XLD) agar plates (MERCK, India). Following incubation for 24 h at 37°C, three to four *Salmonella*-like colonies (red with or without black centre on XLD) were picked with needle and stabbed-streaked in nutrient agar (NA) slant to examine the swarming phenotype of the isolates. The purified *Salmonella*-typical colonies were examined further for their detail biochemical properties to identify presumptive *P. mirabilis* and finally confirmed by API20E (bioMe ´rieux, Durham, NC).

Antimicrobial susceptibility testing (AST)

AST for *P. mirabilis* isolates was done following Kirby-Bauer disk diffusion method [18] and the zone of inhibition were interpreted according to instructions from the Clinical and Laboratory Standards Institute (CLSI, 2010). About 20 different antibiotics were reported to be used frequently by Bangladeshi poultry farmers [4]. Of them, six antimicrobials (ABs) from five generic groups, namely, β-lactam, quinolone, tetracycline, aminoglycosides and synthetic antibiotic (trimethoprim-sulfamethaxole), were tested to characterize *P. mirabilis* in this study. Commercially available antimicrobial discs (Oxoid, UK) and Mueller-Hinton agar (MHA, Oxoid, UK) media were used for the assay.

In this method, *P. mirabilis* isolates grown on XLD were then inoculated into nutrient broth for 18–24 hours at 37°C. Then, one loopful of inocula was added onto 9 mL of MH-broth (Oxoid, UK.) and again incubated aerobically at 37°C but for 5–6 hours only to reach standard turbidity of growth near 10⁸ colony forming unit per milliliter (CFU/mL). The inocula were then lawned evenly using sterile cotton swabs on MHA plates. After air drying (under a safety hood), all the 6 AB-discs such as ampicillin (AML) 10 µg, ciprofloxacin (CIP) 5 µg, gentamycin (GN) 10 µg, nalidixic acid (NA) 30 µg, tetracycline (TE) 30 µg and trimethoprim- sulfamethoxazole (SXT) 25 µg were placed on MHA aseptically and kept at 4°C for 30–60 minutes for adequate diffusion. The plates containing AB-discs were incubated overnight at 37°C keeping in upright position and the diameter of zone of inhibition were read to interpret as resistant, intermediate/moderately sensitive, sensitive (susceptible) according to the reference inhibition zone by respective antibiotic. Reference non-pathogenic *Escherichia coli* (*E. coli*) were used as control strain.

Results

Isolation and confirmation of *Proteus mirabilis*

To isolate and identify *Proteus mirabilis*, a sum of 70 chicken droppings were collected from Savar area of Bangladesh (Figure 1). Colony characteristics of *P. mirabilis* on XLD medium (red with black centre colonies), were similar to those of *Salmonella spp.*, whereas the negative control, *E. coli* showed different colony characteristics; yellowish to white without black-centre on XLD. When we examined

Antimicrobial agent	No. (%) of sensitive ^a isolates, n=36	No. (%) of moderate sensitive isolates, n=36	No. (%) of resistant isolates, n=36
Ampicillin	10 (27.8)	2 (5.6)	24 (66.7)
Ciprofloxacin	18 (50)	12 (33.3)	6 (16.7)
Gentamicin	15 (41.6)	2 (5.6)	19 (52.8)
Naladixic acid	3 (8.3)	1 (2.8)	32 (88.9)
Tetracycline	1 (2.8)	1 (2.8)	34 (94.4)
Trimethoprim+	8 (22.2)	4 (11.1)	24 (66.7)
Trimethoprim-Sulfamethaxole			

Table 2: Antimicrobial susceptibilities^a of *P. mirabilis* isolated from chicken droppings.

^aAntimicrobial susceptibility testing for *P. mirabilis* isolates was done following Kirby-Bauer disk diffusion method.

^bResults were interpreted according to instructions from the clinical and laboratory standards institute (CLSI, 2010).

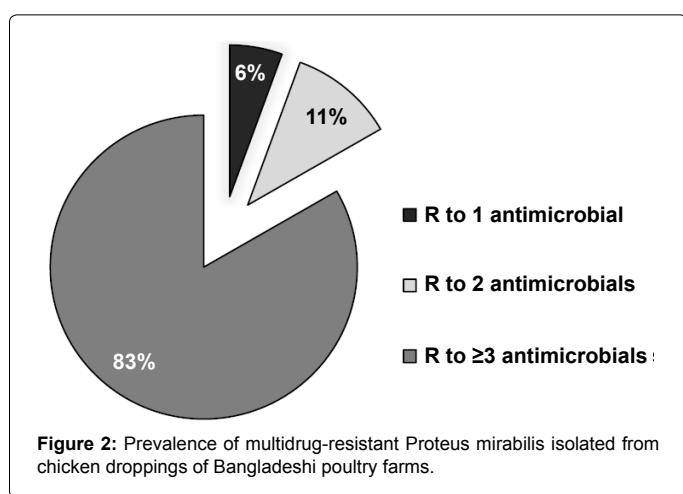


Figure 2: Prevalence of multidrug-resistant *Proteus mirabilis* isolated from chicken droppings of Bangladeshi poultry farms.

those 81 *Salmonella* like colonies (on XLD), 36 (44%) were identified as *P. mirabilis* based on their detailed biochemical properties (slant: alkaline/red, and butt: acidic/yellow, producing H₂S in Kligler Iron Agar [KIA] test). All those were oxidase negative, urease positive; and catalase positive. Moreover, these isolates showed typical biochemical markers of 'IMViC' as '- + - +/-' (indicates: indole negative; methyl red positive; VP positive/negative and citrate variable [+/-]). Under light microscopy, these showed gram-negative short rod (under 100x oil emersion magnification). Isolates of *P. mirabilis* were further confirmed by the API 20E system.

The isolation rates of *Proteus mirabilis* in tested poultry samples

This study revealed the presence of *P. mirabilis* in samples of chicken droppings in five of seven farms; therefore, the overall farm prevalence for *P. mirabilis* became 71% (Table 1). All the 20 samples (ID CK001 to CK020) collected from PF1 (broiler type) and PF2 (layer type) chicken, both being located at 'South Rajason' area, showed growth of plentiful droppings-typical bacteria, but not *P. mirabilis*. In contrast, samples from other PFs yielded *P. mirabilis*, with intra-sample presence of 30% in PF4 to 90% in PF7 (Table 1). Thus, the overall presence among 70 samples of chicken droppings yielded 38.6% *P. mirabilis* (27/70). We tested 36 isolates from 27 culture-positive samples for AST.

Antimicrobial resistance patterns (AST) of *Proteus mirabilis*

The pattern of AST for 36 *P. mirabilis* isolates varied in its susceptibility/resistance pattern using six different AB-disks belonging

to five different generic groups. The antimicrobial resistance profile showed tetracycline as the highest resistant AB (94.4%; 34 of 36) followed by nalidixic acid (89%, 32 of 36) (Table 2). Isolates showing higher level of resistance to β -lactam antibiotic were ampicillin (66.7%, 24 /36) and trimethoprim-sulfamethaxole (66.7%, 24/36). Similarly, aminoglycoside (Gentamycin) were resistant to 53% (19 of 36 isolates). In contrary, ciprofloxacin revealed relatively lower resistance (16.7%, 6 of 36) (Table 2). Combining the results together, we observed 83.3% *P. mirabilis* (30 of 36) were found resistant to 3 or more ABs, followed by 11% isolates (4/36) showing resistance to 2 ABs evidencing multidrug-resistant (MDR) (Figure 2). However, only 5.6% (2 of 36) were found to be resistant to single AB. Alarming, no isolate was found to be susceptible to all AB tested.

Discussion

Ever growing global migratory trend, rapid industrialization and extensive growth in poultry production are thought to contribute the possible rapid dissemination of zoonotic pathogens posing public health a potential concern [19-21] and a big threat. Few studies have been reported from Bangladesh on the zoonotic infections among poultry, despite rampantly expansion of the PF industries [2,4,5].

In spite of indispensable mandate to follow stringent biosecurity guidelines to limit and prevent transmission of potential pathogens via poultry products [22,23], the benchmark guidelines of biosecurity are often not practiced in majority of PFs in Bangladesh as latest reports revealed [12]. This factor plausibly contribute in transmitting potential zoonotic pathogens to PF workers directly (while handling fecal contaminated meat/eggs) or indirectly (while processing/dressing infected poultry) [19-21] which ultimately spread out among the catchment population and surrounding environment. Evidences from latest reports show that poultry-borne *Proteus spp* were associated with zoonotic UTI [24] and that lead to rheumatoid arthritis (RA) very commonly in developing countries [25], with particularly on *P. mirabilis* causing nosocomial/ wound infections [15].

Findings of our study (38.6% *P. mirabilis* in chicken droppings) remain a potential public health concern in poultry industry of Bangladesh. This study has covered small number of poultry farms from focal area and detected 71% farm-prevalence for *P. mirabilis* and thereby has generated the baseline prevalence-data for the bacteria in the poultry industry of this country. The outcomes of this study would be helpful in designing further extensive investigations covering more poultry farms from expanded areas of the country to find nationwide prevalence of this zoonotic human pathogen. Emerging of MDR poultry-origin *P. mirabilis* has increased over the recent years [8,10,16,26], remains comparable to that of ours. In this study, isolates from chicken droppings showed high resistance properties towards tetracycline, nalidixic acid, ampicillin trimethoprim-sulfamethaxole and gentamycin. Individually, the high resistant of *P. mirabilis* to individual AB like tetracycline does attest the findings of earlier study [27]. More importantly, emergence of resistance against β -lactam antibiotic (we studied here ampicillin only) becoming alarming, since β -lactams are often remain the typical choice by the clinicians in treating a wide range of infections caused by *Proteus spp*. as a recent report says [9]. We believe that a more detailed study on extended-spectrum β -lactam (ESBL) antimicrobials would assist further in clarifying issue on emergence of resistance by *P. mirabilis*. However, we observed, unlike other antimicrobials, ciprofloxacin, a quinolone antimicrobial revealed much better potency (>80% were susceptible) against *P. mirabilis*.

We postulate several plausible factors, like environmental degradation (grossly polluted), disease profile (much higher prevalence of communicable/infectious disease), natural disasters (round the year flood, or cyclone, or tidal bores, etc.) may have augmented in the emergence of MDR issues directly or indirectly. In Bangladesh, indiscriminant use of AB (either irrational prescription by unqualified village-doctors/quacks or improperly taking AB or non-compliance of correct AB-dosage taken by the patients) and overuse/misuse of easily available AB by the PFs/allied industries to prevent their poultry-flocks from unexpected diseases or deaths. Therefore, the acquisition of antimicrobial resistance may occur due to the selective pressure of AB abuses.

The drug-administration approved antimicrobial compounds are easily available in open markets to treat broiler/layer chickens in Bangladesh. These include ciprofloxacin, streptomycin, gentamicin, erythromycin, tetracycline, furazolidone and many others [4]. These are used singly or in combination with 2 or more ABs, which has been reported to contribute significantly in the emergence of MDR-resistance in chicken isolates [28]. We therefore postulate further, that high percentages of MDR *P. mirabilis* in chicken droppings that we observed here, may threaten the total public health in Bangladesh at large through spreading that out among adjacent communities (oral-fecal route of contamination). This may also be augmented by common unhygienic defecation practice (with inadequate post-toileting hand washing) by the vast majority rural inhabitants hindered by grossly inadequate water and sanitary system in Bangladesh.

High population density of Bangladesh may also contribute in spreading *P. mirabilis* following person to person transmission (PF environment/community to adjacent healthy populations) much faster to contaminate the surrounding population. This remains more true to hospital environment where this might subsequently result in nosocomial mediated *P. mirabilis* infections, as reported by Ebringer et al. [25]. Staying apart from the aforementioned postulates, a more logical stronger pre-hypothetical nod exists in chick-guts: MDR *P. mirabilis* in the gut of chicken may favor well in transmitting MDR genetic traits dangerously to AB-susceptible- *P. mirabilis* strain even to other Gram-negative gut microbiota through interspecies horizontal transfer as evidenced earlier [29,30]. More studies will be needed to evaluate and transmission of the antimicrobial resistance via poultry borne *P. mirabilis*. The resistant pattern of poultry originated *P. mirabilis* has not been compared and standardized with that of human clinical isolates. Establishment of clonal relationship between these poultry and clinical isolates would be noteworthy to translate our preliminary observation in PF into human-clinical applications, more effectively. We strongly recommend further large scale multi-center research encompassing larger sample size, involving heterogenic poultry farms from diversified areas to examine details of phenotypic and genotypic characterization of this zoonotic pathogen *P. mirabilis*.

Conclusion

To our knowledge, it is the first study of its type describing the presence of *Proteus mirabilis* in Bangladeshi poultry samples. We believe the data that this study generated will contribute to serve as the baseline information on the emerging communicable diseases in PFs in Bangladesh. Our finding demands stringent surveillance system to be developed in Bangladesh for antimicrobial resistance monitoring and biosafety on *P. mirabilis* and other pathogens found in poultry products.

Author Contributions

AN, collected samples, performed major experiments, prepared the results and helped in manuscript preparation; MS and SIA, helped sample-collection, assisted laboratory experiments and data acquisition; SN, contributed reagents and assisted data analysis; KSA, conceived the research idea, editing of first two drafts of this report and finalized the manuscript; SI, conceived, designed and coordinated the experiments, wrote the manuscript.

Competing Interests

None of the authors declared competing or conflict of interest

Ethics Statement

Verbal consents were obtained from farm owners for collection of respective chicken droppings and anonymity was strictly maintained.

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References

1. FAO (2013) Food and Agriculture Organization of the United Nations.
2. Nandi SP, Sultana M, Hossain MA (2013) Prevalence and characterization of multidrug-resistant zoonotic Enterobacter spp. in poultry of Bangladesh. *Foodborne pathogens and disease* 10: 420-427.
3. Conan A, Goutard FL, Sorn S, Vong S (2012) Biosecurity measures for backyard poultry in developing countries: a systematic review. *BMC veterinary research* 8: 240.
4. Hasan B, Faruque R, Drobní M, Waldenstrom J, Sadique A, et al. (2011) High prevalence of antibiotic resistance in pathogenic *Escherichia coli* from large- and small-scale poultry farms in Bangladesh. *Avian diseases* 55: 689-692.
5. Barua H, Biswas PK, Talukder KA, Olsen KE, Christensen JP (2014) Poultry as a possible source of non-typhoidal *Salmonella enterica* serovars in humans in Bangladesh. *Veterinary microbiology* 168: 372-80.
6. Musgrove MT, Northcutt JK, Jones DR, Cox NA, Harrison MA (2008) Enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. *Poultry science* 87: 1211-1218.
7. King MD, Guentzel MN, Arulanandam BP, Lupiani B, Chambers JP (2009) Proteolytic bacteria in the lower digestive tract of poultry may affect avian influenza virus pathogenicity. *Poultry science* 88: 1388-1393.
8. Kim SH, Wei CI, An H (2005) Molecular characterization of multidrug-resistant *Proteus mirabilis* isolates from retail meat products. *Journal of food protection* 68: 1408-1413.
9. Wong MH, Wan HY, Chen S (2013) Characterization of multidrug-resistant *Proteus mirabilis* isolated from chicken carcasses. *Foodborne pathogens and disease* 10: 177-181.
10. Tonkic M, Mohar B, Sisko-Kraljevic K, Mesko-Meglic K, Goic-Barisic I, et al. (2010) High prevalence and molecular characterization of extended-spectrum beta-lactamase-producing *Proteus mirabilis* strains in southern Croatia. *Journal of medical microbiology* 59: 1185-1190.
11. Lima-Filho JV, Martins LV, Nascimento DC, Ventura RF, Batista JE, et al. (2013) Zoonotic potential of multidrug-resistant extraintestinal pathogenic *Escherichia coli* obtained from healthy poultry carcasses in Salvador, Brazil. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases* 17: 54-61.
12. Barua H, Biswas PK, Olsen KE, Shil SK, Christensen JP (2013) Molecular characterization of motile serovars of *Salmonella enterica* from breeder and commercial broiler poultry farms in Bangladesh. *PLoS one* 8: e57811.
13. Cheng W, Chen H, Su C, Yan S (2013) Abundance and persistence of antibiotic resistance genes in livestock farms: a comprehensive investigation in eastern China. *Environment international* 61: 1-7.

14. Mellata M (2013) Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne pathogens and disease* 10: 916-932.
15. Jacobsen SM, Stickler DJ, Mobley HL, Shirliff ME (2008) Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clinical microbiology reviews* 21: 26-59.
16. Aragon LM, Mirelis B, Miro E, Mata C, Gomez L, Rivera A, Coll P, Navarro F (2008) Increase in beta-lactam-resistant *Proteus mirabilis* strains due to CTX-M- and CMY-type as well as new VEB- and inhibitor-resistant TEM-type beta-lactamases. *The Journal of antimicrobial chemotherapy* 61: 1029-1032.
17. Frands Dolberg (2008) Poultry sector country review: Bangladesh.
18. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology* 45: 493-496.
19. Le Hello S, Hendriksen RS, Doublet B, Fisher I, Nielsen EM, et al. (2011) International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. *The Journal of infectious diseases* 204: 675-684.
20. Beeckman DS, Vanrompay DC (2009) Zoonotic *Chlamydia psittaci* infections from a clinical perspective. *Clinical microbiology and infection* : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 15:11-17.
21. WHO (2011) Influenza at the human-animal interface.
22. Defra (2005) Biosecurity and preventing disease –Peace of mind, a healthier flock and a more viable business.
23. FAO (2006) Guide For The Prevention And Control Of Avian Flu In Small Scale Poultry.
24. Armbruster CE, Smith SN, Yep A, Mobley HL (2014) Increased Incidence of Urolithiasis and Bacteremia During *Proteus mirabilis* and *Providencia stuartii* Coinfection Due to Synergistic Induction of Urease Activity. *The Journal of infectious diseases* 209: 1524-1532.
25. Ebringer A, Rashid T (2014) Rheumatoid arthritis is caused by a *Proteus* urinary tract infection. *APMIS* 122: 363-368.
26. Park SD, Uh Y, Lee G, Lim K, Kim JB, et al. (2010) Prevalence and resistance patterns of extended-spectrum and AmpC beta-lactamase in *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Salmonella* serovar Stanley in a Korean tertiary hospital. *APMIS* 118: 801-808.
27. Magalhaes VD, Schuman W, Castilho BA (1998) A new tetracycline resistance determinant cloned from *Proteus mirabilis*. *Biochim Biophys Acta* 1443: 262-266.
28. McEwen SA, Fedorka-Cray PJ (2002) Antimicrobial use and resistance in animals. *Clinical infectious diseases* 34 Suppl 3: S93-S106.
29. Leverstein-van Hall MA, Box AT, Blok HE, Paauw A, Fluit AC, et al. (2002) Evidence of extensive interspecies transfer of integron-mediated antimicrobial resistance genes among multidrug-resistant Enterobacteriaceae in a clinical setting. *The Journal of infectious diseases* 186: 49-56.
30. Butaye P, Michael GB, Schwarz S, Barrett TJ, Brisabois A, White DG (2006) The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. *Microbes Infect* 8: 1891-1897.