**Title:** Prevalence of selected zoonotic food-borne pathogens of chickens in south and south-east Asia: *A systematic review and meta-analysis*

**Introduction:**

Diarrheal diseases are serious health problems that cause high rates of morbidity and mortality in developing countries (Ayed, [2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6145270/#fsn3725-bib-0001)). *Campylobacter, Salmonella* and *E. coli* are the main pathogens implicated in these diarrheal diseases worldwide (Mir et al., [2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6145270/#fsn3725-bib-0017)). Poultry is one of the principal asymptomatic carriers of *Campylobacter*, *Salmonella* and *E. coli* O157; and the process of removing the gastrointestinal tract during slaughtering is regarded as one of the most important sources of carcass and organ contamination with these pathogens. Cross‐contamination may occur during the preparation of these carcasses, increasing the risk of contamination for the consumers (Mir et al.,2015; Zhang et al., 2016).

Since the early 1990s, *Salmonella* strains have played an important role in infectious diseases. They are responsible for a large number of food poisoning infections, and recently, the appearance of multi-drug resistant strains has increased concern (Kagambèga et al., 2018). According to the Surveillance for Foodborne Disease Outbreaks of the United States, 11.2% of the outbreaks caused by *Salmonella* with a confirmed serotype were associated with the consumption of poultry meat in the period of 1998-2008, and *Salmonella* and poultry was considered to be the most commonly responsible pathogen-commodity pairs for outbreaks caused by bacteria. More surprisingly, isolated *Salmonella* serovars have been found resistant to three or more classes of antimicrobial agents (referred to here as multidrug-resistant, MDR) (Magiorakos et al., 2012), thereby increasing the risk of treatment failure when use of these agents is indicated (Aminov, 2010, Bai, Zhao et al., 2016; Bai, Hurley et al., 2016).

*Campylobacter* strains have been recognized as a major cause of bacterial gastroenteritis in humans since 1970 and are responsible for 400–500 million cases of diarrhea each year worldwide (Ruiz‐Palacios, [2007](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6145270/#fsn3725-bib-0024)). In developing countries, the incidence among children under five years old is estimated as 40,000 cases per 100,000 per year (Oberhelmanand and Taylor, [2000](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6145270/#fsn3725-bib-0020)). According to the World Health Organization, this incidence is underestimated (WHO, 2001). Poultry, mainly chickens, are considered important reservoirs of these microorganisms, but they rarely show signs of clinical disease (Horrocks et al., [2009](https://www.tandfonline.com/doi/full/10.1080/03079457.2012.734915)). The broiler reservoir as a whole and the handling, preparation and consumption of broiler meat have been reported as the main sources of human campylobacteriosis in the European Union (EFSA, [2010a](https://www.tandfonline.com/doi/full/10.1080/03079457.2012.734915)).Since the 1990s an increasing resistance to these antimicrobials has been observed in *Campylobacter* isolates (Luangtongkum et al., [2009](https://www.tandfonline.com/doi/full/10.1080/03079457.2012.734915)), and food of animal origin may represent a vehicle of transmission of resistant *Campylobacter* strains to humans (Aarestrup et al., [2008](https://www.tandfonline.com/doi/full/10.1080/03079457.2012.734915)).

*E. coli*, a natural inhabitant of the human intestinal tract and warm-blooded animals, is used as an indicator bacterium because these bacteria acquire antimicrobial resistance faster than other conventional bacteria (Miranda et al., 2008). According to WHO (2004), diarrheal diseases caused by *E. coli* account for more than 4% of the total daily global disease burden every day and about 1.8 million deaths occur every year, of which 90% are children. As poultry is a potential carrier of *E. coli,* the risk of illness through contamination of chickens with pathogenic *E*. *coli* should be a concern for all parties from farm to fork. The prevalence of AMR *E*. *coli* isolates has increased in low- to moderate-income countries, likely as the result of the very liberal and uncontrolled use of antibiotics, to the extent that it is becoming a threat to medical and veterinary treatment efficacy (Osman et al., 2018).

However, the sources and transmission routes of *Campylobacter, Salmonella and E. coli* in developing countries are poorly understood due to the lack of coordinated national epidemiological surveillance systems (Kariuki et al., 2006; Kagambègaet al., [2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6145270/#fsn3725-bib-0011)).

People infected with antimicrobial resistant bacteria are more likely to suffer an adverse health event such as prolonged illness, increased severity of illness, hospitalization or death when compared with those infected with susceptible isolates (Hu et al., 2017). So, food safety regarding zoonotic food-borne pathogens and their antimicrobial resistance is very crucial.

**Aims and objectives:**

This study aims to perform a systematic review on the detection, prevalence of and risk factors associated with *Salmonella, Campylobacter and E. coli* in chickens and chicken products to inform food safety policy and identify data gaps. This aim is met via the following objectives:

1. Assessing the detection and/or prevalence of non-typhoidal *Salmonella* spp, *Campylobacter* spp *and E. coli* in chickens/chicken meat/eggs/processed products in south and south-east Asia.
2. Identification of the factors associated with the presence of non-typhoidal *Salmonella* spp, *Campylobacter* spp *and E. coli* in chickens/chicken meat/eggs/processed products in south and south-east Asia.
3. Assessing the effect of intervention measures (including modification or removal of risk factors) on the occurrence of non-typhoidal *Salmonella* spp, *Campylobacter* spp *and E. coli* in chickens/chicken meat/eggs/processed products in the field in south and south-east Asia.
4. Identification of the potential research gaps of the pathogens in chickens and chicken products in south and south-east Asia.

**Criteria to select articles for review:**

* Published between 2000- May 2020
* Published research articles (full length/ short communication), grey literature, reports and unpublished data
* Observational studies and field trials that should include at least the total number of chickens/chicken meat/eggs/ processed products being tested and the number of positive samples
* Articles had to describe the identification, or prevalence of non-typhoidal *Salmonella* spp, *Campylobacter* spp and *E. coli* in chickens and/or chicken meat/eggs/processed products in south and south-east Asia
* Articles that described the effectiveness of intervention measures (through observational studies and field trials) to reduce the prevalence of the set pathogens or identified the risk factors associated with the set pathogens in chickens/chicken meat/eggs/processed products in south and south-east Asia
* The diagnostic methods for non-typhoidal *Salmonella* spp, *Campylobacter* spp *and E. coli* had to be standard bacteriological culture/any molecular technique
* Articles are excluded if the titles, abstracts or full text did not fulfill the set criteria

**Databases selected:**

1. PubMed: 481\_tit/abs\_22 june\_19c\_All lan
2. Scopus
3. HINARY: 7137\_full text\_23 june
4. CABI:
5. Web of Science: 406\_Topic\_23 june
6. Google for grey literature search

**Description of example (non-typhoidal *Salmonella* spp) search syntax/term:**

Salmonell\*

AND

Chicken OR chickens OR Broiler OR layer OR poultry OR hen OR cock OR cocks OR cockerel OR laying hen OR chick OR chicks

(Chicken) OR (chickens) OR (Broiler) OR (layer) OR (poultry) OR (hen) OR (cock) OR (cocks) OR (cockerel) OR (laying hen\*) OR (chick) OR (chicks)

AND

South Asia\* OR Southeast Asia\* OR Afghanistan\* OR India\* OR Pakistan\* OR Bangladesh\* OR Sri Lanka\* OR Nepal\* OR Bhutan\* OR Maldives\* OR Indonesia\* OR Malaysia\* OR Singapore\* OR Philippines\* OR East Timor\* OR Brunei\* OR Cambodia\* OR Laos\* OR Lao OR Myanmar OR Burma\* OR Thailand\* OR Vietnam\* OR Viet Nam

(South Asia\*) OR (Southeast Asia\*) OR (Afghanistan\*) OR (India\*) OR (Pakistan\*) OR (Bangladesh\*) OR (Sri Lanka\*) OR (Nepal\*) OR (Bhutan\*) OR (Maldives\*) OR (Indonesia\*) OR (Malaysia\*) OR (Singapore\*) OR (Philippines\*) OR (East Timor\*) OR (Brunei\*) OR (Cambodia\*) OR (Laos\*) OR (Lao) OR (Myanmar) OR (Burma\*) OR (Thailand\*) OR (Vietnam\*) OR (Viet Nam)

**Limits:**

Year- 2000- May 2020

Language- English

Field- title/abstract/keywords

**Flow chart: Following the PRISMA guidelines**

**Tasks to follow:**

1. Run searching with search items (Nurun to do)

2. Remove duplicate articles (Nurun to do)

3. Draft an excel template to record articles according to the titles

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Authors** | **Title** | **Year** | **Journal** | **Inclusion (yes/no)** | **If No, reason (Not targeted pathogens/ species (chicken)/area/ study type/ other)**  | **If Other, reason** | **Comment** |
|  |  |  |  |  |  |  |  |  |

4. Draft an excel template to record articles according to abstracts of short-listed articles

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SN** | **Authors** | **Title** | **Year** | **Journal** | **Inclusion (yes/no)** | **If No, reason (Not targeted pathogens/ species (chicken)/area/ study type/ other)**  | **If Other, reason** | **Comment** |
|  |  |  |  |  |  |  |  |  |

6. When there is a disagreement on exclusion (title/abstract), discussion between reviewers can solve the problem. If the disagreement cannot be solved based on the title (screening-1) / or the abstract, screening-2), then the article is shortlisted, to the next stage.

7. Download the full selected articles (several peoples, at least one from each country will help do this)

8. Draft the data extraction sheet and decide how to assess quality of each article (study)

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|  |
| **Domain** | **Explanation** |
| Bias due to confounding | Baseline confounding occurs when one or more prognostic variables (factors that predict the outcome of interest) also predicts the exposure received at baseline.Time-varying confounding occurs when individuals switch between the interventions/exposures being compared and when post-baseline prognostic factors affect the exposure received after baseline. |
| Bias in selection of participants into the study | When exclusion of some eligible participants, or the initial follow-up time of some participants, or some outcome events is related to both interventions/risk factor, there will be an association between interventions/risk factor and outcome even if the effects of the interventions/risk factors are identical.This form of selection bias is distinct from confounding—A specific example is bias due to the inclusion of prevalent users, rather than new users, of an intervention. |
| Bias in measurement of exposures | When exposures are not well deﬁned or information on exposures are affected by knowledge of the infection status or risk of infection. |
| Bias due to missing data | Bias that arises when later follow-up is missing for individuals initially included and followed (such as differential loss to follow-up that is affected by prognostic factors); bias due to exclusion of individuals with missing information about exposure status or other variables such as confounders. |
| Bias in measurement of outcomes | Bias introduced by either differential or non-differential errors in measurement of outcome data. Such bias can arise when outcome assessors are aware of exposure status, if different methods are used to assess outcomes in different exposed groups, or if measurement errors are related to exposure status or effects. |
| Bias in selection of the reported result | Selective reporting of results in a way that depends on the findings and prevents the estimate from being included in a meta-analysis (or other synthesis). |

8. Draft an excel template to record articles short-listed articles after reading the full text (by R-1-Nurun: BD and R-2: GR/TN/SR/VN/UK in parallel)

10. Screen the full articles (Several peoples, at least one from each country will help do this)

12. Data extraction and entry (Nurun and one more person, independently)

13. Review the data sheet (It will depend on whether we decide to have one paper or one/country)

1. Data analysis (Nurun to do)

15. Manuscript writing (Nurun and others) (If multiple manuscripts, then we need to follow different approaches.)