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Systematic Review: Update on Identification and Characterization Methods and Antibiotic Resistance Profile of Enteropathogenic Bacteria Isolated from Food: Case of *Salmonella*

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ABSTRACT:

Foodborne illness has a significant impact on public health. The emergence of *Salmonella* is now a global challenge in the public health and food production sector. This review aims to provide an update on methods for isolating and characterizing *Salmonella* in food and their antibiotic resistance profile. Thus, this synthesis is carried out according to the method "*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*" (PRISMA). One hundred and sixty-nine (169) publications were found. From this range of publications, twenty-five (22) were selected after a number of selection criteria were applied. The result of this review shows that the detection of *salmonella* in food is carried out in three (3) stages (pre-enrichment, enrichment and isolation). Thus, several methods are used for characterization, some biochemical and others molecular (reduced Leminor rack, API 20E, PCR). Indeed, several strains of *salmonella* have been shown to be resistant to certain types of antibiotics. The most frequently encountered resistances are for penicilin (56.5%), Tetracycline (56.5%), Streptomycin (30.4), Trimethoprim + Sulfamethoxazole (30.4%), Amoxicilin + clavulanic acid (26.1%), Gentamycin (13%), Kanamycin (13%), Cefalotin (13%), Chloramphenicol (13%). The results of this analysis are a step in understanding conventional and modern methods used to test for salmonella in food and a good guide to the best choice of antibiotics.

Keywords: *Salmonella*, food, isolation, characterization, antibioresistance profile

Introduction

Every day, we ingest a large number of microorganisms in our food. Due to their microscopic size, they are invisible to the naked eye and therefore represent an invisible danger. A minority of these microorganisms are pathogenic [1]. One of the most feared incidents in the Food and Food industry is the contamination of food by pathogenic enteric microorganisms (bacteria, viruses, parasites). For a long time, it was thought that pathogenic enteric bacteria, emitted into the environment via faecal discharges (treatment plants, septic tanks, slurry spreading, pastures, etc.), had only a very limited lifespan [2]. To fully understand the action of pathogenic microorganisms, it is first necessary to know their properties in food. These organisms are present everywhere and are multiplying rapidly. Human foods are exceptional growing media for microorganisms in general [1]. Foodborne zoonoses have a major impact on public health. Bacterial contamination of food is more frequent, either of animal origin (red meat, poultry, etc.) or following handling, these contaminations are transferred to humans [3].

Collective foodborne infection accounts for a considerable number of deaths worldwide in developing countries. It is estimated that pathogenic microorganisms in food are responsible for approximately 6.5 to 33 million toxi-infections and more than 9000 human deaths per year worldwide [4]. However, pathogenic enteric bacteria, such as *Salmonella* and some enterohaemorrhagic serotypes of *Escherichia coli,* are implicated in an increasing number of collective foodborne infections [2]. The majority of reported cases of microbial toxi-infection (95%) are caused by food prepared at home, in restaurants or in institutions. An estimated 5% of cases are caused by food produced in industry [1]. *Salmonella* is one of the main causes of collective foodborne illness in developing countries [5,6,7]. In human pathology, *Salmonella* is divided into typhic serotypes (S. Typhi and S. Paratyphi) and Non-Typhic serotypes [8]. Non-typhoid *salmonella* is the basis of salmonellosis by

contaminated food products such as fresh produce, eggs, pork, vegetables and seafood [9,10,11]. Fruits and vegetables have also been reported as a vector of transmission and contamination can occur at several stages of the food chain [12].

In addition, antibiotics and antibiotic products are commonly used for therapeutic, prophylactic and zootechnical (growth stimulator) purposes. Thus, their misuse leads to the selection of resistant germs [13]. The International Network of Food Safety Authorities has reported that misuse and uncontrolled administration of antibiotics have led to the selection of resistant bacteria [14,15]. The objectives of this synthesis will be to (1) review the different methods of identification and characterization of *Salmonella* in food and (2) describe the antimicrobial resistance profile of this germ.

1. Research methodology

The inclusion and exclusion method used is based on the PRISMA method "*Preferred Reporting items for systematic Reviews and Meta-Analyses*". Each section of the review was designed and reported in accordance with PRISMA guidelines [16].

1.1 Search Strategy

The bibliographic search is carried out on the search engine "Google" "Google Scholar" and on "Pubmed". The aim is to identify methods for identifying and characterising and antimicrobial resistance profiles of foodborne *salmonella*. The terms used in the search engines were: "*Salmonella*, Food *Salmonella*, *Salmonella* antibiotic resistance, *Salmonella* characterization".

1.2 Study selection

All articles identified from the different databases have been imported into the reference manager (Excel file). After the removal of duplicates, three levels of selection based on title, abstract and full-text review were performed [16]. Articles with only obtained titles were excluded from the analysis, as were abstracts and full texts whose authors worked only on specific serotypes. Abstracts and full texts that have been collected multiple times have been excluded [17].

1.3 Eligibility criteria

A study is eligible for this review if:

- It focuses on the study of the strain of *salmonella* in food;
- The identification and characterization methods and the antibiotic resistance profile are well described;
- Its purpose is not to study a specific subspecies or serotype;
- Written in French or English.

1.4 Data extraction

For any selected study, the following data are collected and recorded in an Excel table. These are: the first author, the year of publication of the article, the country where the study was carried out, the species studied, the host studied, the identification and characterization techniques used, the antibiotic resistance observed.

2. Results and discussion

2.1 Results

Sixty-nine (169) reference were found from this search. Eight (8) duplicates were found and excluded, nineteen (19) studies with abstracts only and titles were excluded. One hundred and forty-two (142) studies were included, of which ninety-six (96) animal and/or human or environmental studies, six (6) studies conducted on two or more germs other than *Salmonella* were excluded. Of the forty (40) eligible studies, eighteen studies excluded due to lack of data on antibiotic resistance pattern. A total of twenty-two (22) studies are eligible: two (2) theses and twenty (20) articles (fig.1).

Eligibility

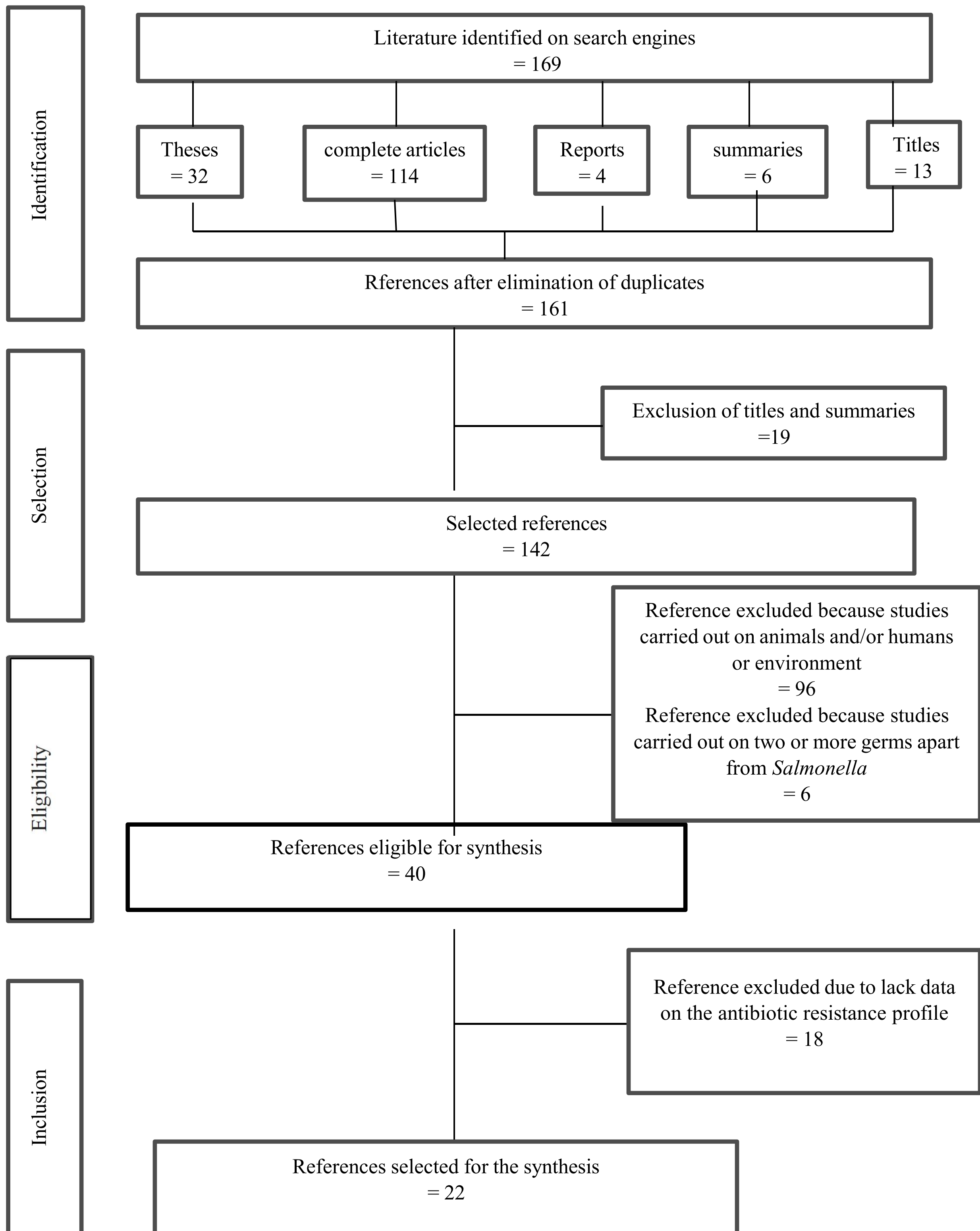

Figure 1: diagram of selected studies

Authors	Pre-enrichment	Enrichment	Isolation	Mini galery	API 20E	PCR
Bouchrif *et al.,* (2008) Morocco	BPW	RVS, SC	XLD	No	Yes	No
Shilangale *et al.,* (2012) Namibia	BPW	RVS, SC, MKTTn	BGA, XLD	No	Yes	No
Kikuvi *et al.,* (2010) Kenya	BPW	MKTTn	DCA	Yes	No	No
Abba *et al.*, (2017) Chad	BPW	RVS, MKTTn	Nr	No	Yes	No
Ammar *et al.*, (2010) Algeria	BPW	RVS, SCS	BGA, Hekt	Yes	Yes	No
El Alloui *et al.*, (2009) Morocco	BPW	RVS, SCS	SS	Yes	No	Yes
Ally *et al.*, (2022) Tanzania	BPW	RVS, MKTTn	BSA, XLD	Yes	No	No
Lynda *et al.,* (2011) Algeria	BPW	RVS, SC	Hekt, XLD	No	Yes	No
Ngai *et al.,* (2021) Kenya	BPW	BPW, SF	SS, XLD	No	Yes	No
Bawa *et al.,* (2015) Burkina Faso	BPW	RVS	Hekt, XLD	No	No	No
Somda *et al.,* (2017) Burkina Faso	BPW	Nr	Nr	No	No	No
Fufa *et al.,* (2017) Ethiopia	BPW	RVS, MKTTn	SS, XLD	Yes	Yes	No
Nwiyi *et al.,* (2018) Nigeria	BPW	RVS	DCA, MC	Yes	No	Yes
Zewdu *et al.*, (2019) Ethiopia	BPW	RVS, MKTTn	BGA, XLD	Yes	No	No
Sarba *et al.,* (2020) Ethiopia	BPW	RVS	BGA, XLD	Yes	No	No
Munuo *et al.,* (2017)Tanzania	SF	SF	BA, BGA, MC	Yes	No	Yes
Tinega *et al.,* (2016) Uganda	BPW	MKTTn	XLD	Yes	No	Yes
Mekonnen *et al.,* (2022) Ethiopia	BPW	RVS, SC	XLD	Yes	No	Yes
Douamba *et al.,* (2022) Burkina Faso	BPW	RVS, MKTTn	SS, XLD	No	Yes	Yes
Anejo-Okopi *et al.,* (2016) Nigeria	BPW	RVS	EMB, SS, XLD	No	No	No
Toe, (2018) Ivory Cost	BPW	RVS, MSRVS	Hekt, SS	No	Yes	Yes
Rachide, (2009) Algeria	BPW	SC, MKTTn	Hekt	No	No	No

BPW: Buffered Peptone Water; RVS: Rappaport Vassiliadis Soya; MKTTn: Muller Kaufman with Tetrathionate novobiochina; SC: Selenite Cysteine; SS: Salmonella-Shigella Agar; XLD: Xylose Dextrose Lysine; Hekt: Hektoen; BA: Blood Agar; BGA: Brilliant Green agar; BSA: Bismuth Sulphate Agar; MC: Mac Conkey Agar; DCA: Desoxycholate agar; EMB: Eosine Methylene Blue; Nr: not defined; SF: Selenite Fecal.

Table 1: Characteristics of eligible studies selected for review

Table 1 presents the conventional techniques used for the isolation (pre-enrichment, enrichment and isolation) and characterization (Mini gallery, API 20E gallery and PCR) of *Salmonella*. Pre-enrichment is reported in almost all studies, performed with Buppered Peptone Water (BPW) as a diluent, except for one study where Selenite Fecal (SF) is used (Munuo *et al.,* 2017). Fifteen (15) studies combined two enrichment techniques (Bouchrif *et al.*, 2008 ; El Alloui *et al.,* 2009 ; Rachide, 2009 ; Ammar *et al.,* 2010 ; Lynda et *al.,* 2011 ; Fufa *et al.,* 2017 ; Alio *et al.,* 2017 ; Toe, 2018 ; Zewdu *et al.,* 2019 ; Ngai *et al.,* 2021 ; Douamba *et al.,* 2022 ; Mekonnen *et al.,* 2022 ; Ally *et al.,* 2022 ;). Seven (7) studies used a single enrichment technique (Kikuvi *et al.,* 2010 ; Bawa *et al.,* 2015 ; Tinega *et al.,* 2016 ; Anejo-Okopi *et al.,* 2016 ; Munuo *et al.,* 2017 ; Nwiyi *et al.,* 2018 ; Sarba *et al.,* 2020) and finally, a single study combined three techniques (Shilangale *et al.,* 2012). Isolation techniques are also many and varied. This review noted the use of XLD, BGA, DCA, BCC, BGA, HEC, BSA, Hekt, SS, MC and EMB for isolation. In this review, eleven (11) studies used the reduced Leminor rack (El Alloui *et al.,* 2009; Kikuvi *et al.,* 2010; Ammar *et al.,* 2010; Tinega *et al.,* 2016; Munuo *et al.,* 2017; Fufa *et al.,* 2017; Nwiyi *et al.,* 2018; Zewdu *et al.,* 2019; Sarba *et al.*, 2020; Mekonnen *et al.,* 2022; Ally *et al.,* 2022).

2.2 Discussion

Isolation and detection methods

Pathogenic microorganisms in food, the environment or faeces are generally present in small numbers and may compete with saprophytic flora, which is abundant in some food matrices [18]. Given the conditions under which food is processed and preserved, bacteria can be stressed. To isolate bacteria, conventional microbiological analysis of food therefore requires several successive steps, resulting in a relatively long response time [19,20,21]. The first step consists of pre-enrichment: the food is homogenized in a rich medium (buffered peptone water, etc.) (eg 25 g of food in 225 ml of diluent) and incubated from 12 to 24 hours. At the end of this step, all the bacteria sought, but also the other bacteria in the sample multiplied [21]. Enrichment is now a question of minimizing the growth of bacteria associated with sampling and promoting the multiplication of the desired bacteria. A selective liquid medium is used and incubation lasts approximately 24 to 48 hours. As for isolation, it is also a selective phase, but on solid medium in Petri dish. The boxes are then incubated for about 24 hours at the appropriate temperature. As for isolation, it is also a selective phase, but on solid medium in Petri dish. The boxes are then incubated for about 24 hours

at the appropriate temperature. Pure strains develop on solid selective medium and are confirmed to belong to the bacterial species sought either by using biochemical galleries or serological tests. This identification can be long (48 hours). The classic diagnosis therefore lasts on average one week. To enumerate germs, the technique consists of inoculating a suspension made from the food on a selective solid medium and counting the characteristic colonies obtained after incubation [1].

Strain Characterization Methods

The identification and classification of *enterobacteriaceae* is based firstly on the study of the following biochemical characteristics, the main ones of which concern mobility, the use of sugars (especially lactose), indole, methyl red, Voges Proskauer (VP), inositol (abandoned) and citrate [22]. These characters are determined according to the Leminor method using Leminor's reduced rack media (Kligler Hajna, Lysine-iron, Simmons Citrate, Mannitol-mobility-nitrate and urea-indole medium). The fermentation of glucose, the oxidation of lactose, the production of gas and hydrogen sulphide (H_2S) is determined on Kligler-Hajna agar; the absence of production of lysine decarboxylase on the lysine-iron medium, the use of citrate as the only source of carbon on Simmons citrate agar, the use of mannitol on mannitol-mobility agar and the production of urease, indole and tryptophan deaminase on Fergusson urea-indole medium. The API 20E gallery is a standardized system for the identification of *Enterobacteriaceae* and other non-fastidious gram-negative bacilli, comprising 20 miniaturized biochemical tests. The API 20E galleries use several types of tests: study of the fermentation of various carbohydrates, auxanogram, direct search for an enzyme. Each tubule contains a different substrate on which the microorganism under consideration will react. They are filled with a calibrated bacterial suspension [23].

Antibiotic resistance profile

Antibiotics are natural or synthetic substances, which can be bacteriostatic or bactericidal at low doses. Their targets of activity are specifically bacterial molecular structures. Therefore, they have selective toxicity to prokaryotic cells and low toxicity to eukaryotic cells [24]. Bacterial resistance is defined as the ability to continue to grow or survive in the presence of the antibiotic. The conditions of activity of an antibiotic are to possess a specific target, to remain in active form, to access the target and to interact effectively with it by deactivating it [22]. In this review, it was noted the use of twenty-nine (29) types of antibiotics belonging to different families (ampicillin, chloramphenicol, trimethoprim, tetracycline, sulfamethoxazole, fusic acid, nalidixic acid, trimethoprim/sulfamethoxazole, gentamycin, kanamycin, amoxicillin + clavulanic acid, ciprofloxacin, spectinomycin, Sulfisoxazole +TR-S, azithromycin, streptomycin, cefotaxime, lincomycin, nitrofurantoin, cefalotin, sulfisoxazole, ceftriaxone, penicillin, co-trimoxazole, ofloxacin, sulfonamide, enrofloxacin, amoxicillin. Several strains of *salmonella* have been shown to be resistant to certain types of antibiotics. The most frequently encountered resistances are for the families of aminopenicillin (penicillin (56.5%), aminoglycosides (gentamycin (13%), kanamycin (13%), streptomycin (30.4)), cephalosporin (cefalotin (13%)), Oxapenams (amoxicillin + clavulanic acid (26.1%)), phenylecylates (chloramphenicol (13%)), tetracycline (tetracycline (56.5%)) and sulfonamides (trimethoprim + sulfamethoxazole (30.4%)).

Table 2: sensibility of *Salmonella* strains to different antibiotics

Antibiotics	Frequency (n)	Percentage (%)
Ampicillin	13	56,5
Tetracycline	13	56,5
Nalidixic acid	8	34,8
Trimethoprim/sulfamethoxazole	7	30,4
Streptomycin	7	30,4
Amoxicillin + clavulanic acid	6	26,1
Chloramphenicol	3	13
Gentamycin	3	13
Kanamycin	3	13
Cefalotin	3	13
Amoxicillin	3	13

The highest pooled sensitivity level in this review is observed for ampicillin and tetracycline (56.5%) for each (table 2). Strong resistance of *salmonella* strains has been reported by Zewdu *et al.,* (2019) (100%); Gebremichael *et al.,* (2019) (89.5%) in Ethiopia for ampicillin. Thus, Fufa *et al.,* (2017) (85.7%) in Ethiopia, Bawa *et al.,* (2015) (78%) in Burkina Faso; Toe, (2018) (61.9%) in Ivory Coast, reported high frequencies of tetracycline. On the other hand, low resistance of around 13% was recorded by Bouchrif *et al.,* (2008) in Namibia for ampicillin and Lynda *et al.,* (2011) in Algeria for tetracycline. Tetracycline resistance is alarming in developing countries and may reflect contamination of raw vegetables with contaminated irrigation water or manure. Moreover, these resistances can potentially be acquired through the food chain from human contamination from therapeutic practices [25,26,27].

In addition, lower levels of susceptibility are observed for nalidixic acid (34.8%), trimethoprim/sulfamethoxazole (30.4%), streptomycin (30.4%), amoxicillin + clavulanic acid (26.1%). Much better results were reported in Ethiopia by Fufa *et al.*, (2017) (81.8% resistance to nalidixic acid) and Sarba *et al.,* (2020) (75% to amoxicillin/clavulanic acid). Then, similar results were reported in Ethiopia by Gebremichael *et al.*, (2019) (31.6% for nalidixic acid), in Burkina Faso by Somda *et al*., (2017) (33% and 36% respectively for trimetroprim-sulfametoxazole and amoxicillin +clavulanic acid).

However, low levels of susceptibility are observed in this review for chloramphenicol, gentamycin, kanamycin, cefalotin, amoxicillin (13% each). Several studies have reported the low resistance of *salmonella* strains to these antibiotics. Such as the study by Bouchrif *et al.,* (2008) (4% for chloremphenicol and 3.8% for kanamycin), Lynda *et al.,* (2011) in Algeria (3.25% for chloremphenicol) and Toe (2018) in Ivory Cost (9.5% for gentamycin).

In addition, in Niger, Alio *et al.,* (2017) reported on lettuce samples, highlights the most frequently encountered resistances to the penicillin A family (ampicillin (27.27%), amoxicillin (11.02%), amoxicillin + clavulanic acid (7.63%)), cephalosporins (ceftazidime (10.17%) and cefepime (11.94%)) and polymyxin (colistin (29.85%)).

Conclusion

This systematic review looked at methods for isolating and characterizing *salmonella* and their antibiotic resistance profiles. The results of this review show three main steps in the isolation of salmonella from food, pre-enrichment in a liquid medium (most often buffered peptone water), enrichment on a selective broth and isolation on a solid medium (either on SS, XLD or Hektoen medium). The characterization method is mainly based on the study of the biochemical characteristics of the strain, however molecular methods based most often on PCR are also used. For the antibiotic resistant profile, *Salmonella* strains were resistant to several types of antibiotics, the most common of which are Aminopenicillins (penicillin), aminoglycosides (gentamycin, kanamycin, streptomycin), Cephalosporin (cefalotin), Oxapenams (amoxicillin + clavulanic acid), Phoenylates (chloramphenicol), tetracycline (Tetracycline) and Sulfonamides (trimethoprim + sulfamethoxazole). This review will allow a better choice of method of isolation and characterization of *salmonella* and also the choice of antibiotics for a good susceptibility test.

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