

***Salmonella* rough strains possessing extended-spectrum
beta-lactamase genes
associated with gastroenteritis of piglets in Meghalaya
(India)**

ABSTRACT

Aim: To study gastroenteritis outbreak in unorganized private pig farm of Meghalaya, a North East State of India.

Materials and Methods: Faecal samples were collected from 10 pigs including 5 severely diarrhoeic piglets and were processed for detection of *Escherichia coli*, *Salmonella*, *Clostridium* spp., *Rotavirus*, as well as parasitic eggs and larvae. The isolates were screen by PCR for rotavirus and presence of virulence genes of *E. coli*, *Salmonella*. Antibiotic sensitivity test was performed on isolated *Salmonella* and screened for presence of extended-spectrum beta-lactamase (ESBL) genes.

Results: A total of 20 *E. coli* were isolated, all of which were found to be negative for any putative virulence genes of shigatoxigenic *E. coli*, enterotoxigenic *E. coli*, enterohaemorrhagic *E. coli* and enteropathogenic *E. coli* pathotypes. From affected piglets, four salmonellae were isolated, which were recorded as *Salmonella* rough strains. All the isolates detected positivity for ESBL (*bla*_{TEM} and *bla*_{CMY-2}) resistance genes. However, no samples were found to be positive for viral and parasitic pathogens under the study. Antibigram study revealed the isolates were resistance to commonly used drugs, such as enrofloxacin, cefalexin, tetracycline, amoxycillin, ampicillin and aztreonam.

Conclusion: The study indicated the involvement of *Salmonella* Rough strains with piglet diarrhoea in an unorganized farm of Meghalaya. Detection of *Salmonella* possessing resistance genes in food animal warranty immediate attention to hygienic practices and regulated use of antibiotics.

Keywords: *Salmonella*, *Rough*, *PCR*, *Drug resistance*, *ESBL*.

1. INTRODUCTION

Salmonellosis is one of the major bacterial diseases affecting both human and livestock species; and porcine due to their scavenging habit are one of the the most affected animal species. *Salmonella* species are important bacterial agents causing many

35 manifestations of enteric and septicaemic disease affecting particularly young ones [1]. So
 36 far, there are more than 2,579 serovars of *Salmonella* that has been recorded with *S. enterica*
 37 subspecies *enterica* contains almost all important pathogenic *Salmonella* serovars [2,3,4].
 38 Among all serovars, the most common serovar of animal origin is *S. typhimurium* followed
 39 by *Salmonella newport* whereas serovars most commonly isolated from human are *S.*
 40 *typhimurium* and *S. enteritidis* [5]. There are many factors contributing to degree of severity
 41 of salmonellosis, most importantly the host condition and the *Salmonella* serovar involved.
 42 Infections in several host species have been attributed to the acquisition and expression of
 43 virulence genes linked to a combination of many chromosomal and plasmid factors [6,7,8].

44 *Salmonella* spp. are usually susceptible to many antimicrobial agents especially to the
 45 third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime but a recent
 46 increase in the rates of resistance to these drugs is of serious concern globally particularly in
 47 developing countries. The present investigation reports the isolation of *Salmonella* rough
 48 strains possessing ESBL genes; from severe diarrhoeic piglets of an unorganized farm in the
 49 tribal area of North East India of Meghalaya.

50 **2. MATERIAL & METHODS**

51 **2.1 Collection of fecal samples**

52 The fecal samples were collected from piglets and growers (3-10 weeks old) suffering
 53 from acute gastroenteritis in an unorganized pig farm of Meghalaya, India during the month
 54 of february 2018. Samples (n=10) were collected from all the animals including five
 55 emaciated diarrhoeic piglets, and transported to laboratory under cold chain.

56 **2.2 Bacteriological, virological and parasitic screening of clinical samples**

57 All the samples were processed for isolation and identification of possible enteric
 58 bacteria including *E. coli*, *Salmonella* and *Clostridium* spp. as per method described by [9].
 59 All the faecal samples were examined for the presence of parasitic eggs and larvae by
 60 standard floatation technique. The faecal samples were also processed for RNA extraction for
 61 screening of *Rotavirus*. In brief, samples were diluted in phosphate buffered saline (pH 7.4)
 62 to prepare a 10% (w/v) faecal suspension. Clarified supernatant was collected and processed
 63 for RNA extraction using Trizol method [10]. Reverse transcription PCR (RT-PCR) targeting
 64 VP7 gene (designed primers) and VP6 gene [11] were used for detection of *Rotavirus* in the
 65 samples.

66 **2.3 Molecular characterization of the isolates**

Detection of putative virulence genes of EPEC (*eaeA*), STEC (*stx1*, *stx2*), EHEC (*hlyA*) was evaluated by multiplex PCR [12] and ETEC (*LT* and *ST*) by [13]. *Salmonella* isolates was subjected to analysis by specific PCR for presence of putative virulence genes *stn* [14], *invA* [15], and ESBL resistant genes *bla*_{TEM} [16], *bla*_{SHV} [17], *bla*_{CMY-2} and *bla*_{CTX-M} [18] as depicted in Table 1. PCR amplification was performed in a Master cycler (Eppendorf, Germany) with PCR mixture in a thin walled 0.2ml PCR tube included a final volume of 25 µl containing 10X Dream taq buffer with MgCl₂ (20mM), dNTP mix (25 mM each), 5 U/µl Taq DNA polymerase, 1µl (20 pmol) each of forward and reverse primer, 4µl of template DNA and nuclease free water to make up the volume 25µl. Amplified products were separated by 1% agarose gel in 1X Tris-borate-EDTA buffer by electrophoresis and stained with ethidium bromide (0.5µg/ml). Standard molecular size marker (100 bp DNA ladder) was included in each gel. DNA fragments were observed and photographed in a gel documentation system (Vilber Lourmat, Gel Imaging system E Box CX5, France).

Molecular typing of the isolates was also performed by randomly amplified polymorphic DNA-PCR (RAPD-PCR) using a single RAPD primer (5'-AAGAGCCCGT-3') according to the method of [19].

2.4 Serotyping of *Salmonella* isolates

The *Salmonella* isolates were serotyped on the basis of their somatic antigen at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh (India).

2.5 Antimicrobial sensitivity assay

The *Salmonella* isolates were subjected to *in vitro* drug sensitivity test by disc diffusion method against 15 commonly used antibiotics (Hi-Media): ampicillin (10mcg), amoxycillin (30mcg), aztreonam (30mcg), cefalexin (30mcg), ceftazidime (30mcg), cefixime (5mcg), ceftriaxone (30mcg), cefotaxime (30mcg), ciprofloxacin (5mcg), enrofloxacin (10mcg), gentamicin (10mcg), imipenam (10mcg), nalidixic acid (30mcg), piperacillin (10mcg) and streptomycin (10mcg). Antimicrobial susceptibility test was done on Mueller-Hinton agar (Hi-Media) plate as per criteria of Clinical Laboratory Standard Institute [20]. Zones of inhibition were measured using zone size interpretative chart furnished by the manufacturer.

3. RESULTS AND DISCUSSION

All the five piglets were suffering from acute gastroenteritis with non haemorrhagic diarrhoea and fever. However, the adults were apparently healthy without any clinical signs. The affected piglets have not received any treatment against any infection till the date of sample collection. There was no report of death of any piglets. On laboratory examination as stated earlier for detection of parasitic and viral pathogens, it was found that no samples contain parasitic eggs or larvae. *Rotavirus* also could not be detected by RT-PCR. In case of bacterial isolation, no clostridial organisms could be detected by anaerobic culture. A total of 20 *E. coli* were isolated and identified from all the 10 samples under the study. On the other hand, five *Salmonella* were isolated and identified from the five diarrhoeic piglets based on standard bacteriological and biochemical tests. On molecular detection of bacterial virulence genes; all the *E. coli* isolates were found to be negative for *eaeA*, *stx1*, *stx2*, *hlyA*, *la* and *st* genes by PCR.

Serotyping of *Salmonella* isolates revealed that four isolates belonged to Rough strains, however, one isolate could not be typed due to report of contamination probably during long distance and other factors. PCR amplification of virulence genes in study revealed all the four *Salmonella* isolates yielded a 1080bp product in the *bla*_{TEM} and 462bp product in the *bla*_{CMY-2} gene segment (Fig.1). However, *stn* and *invA* gene could not be detected in any *Salmonella* isolates.

All the four rough strains showed identical banding patterns when subjected to RAPD-PCR indicating the homogeneity of the strains associated in the disease outbreak. All the *Salmonella* isolates in the present study showed resistance to many antimicrobial drugs used commonly in the region including cefalexin, amoxycillin, ampicillin, enrofloxacin, piperacillin, tetracycline and aztreonam; but were sensitive to ceftazidime, cefixime, gentamicin, cefotaxime, nalidixic acid, streptomycin, ciprofloxacin, ceftriaxone and imipenem.

Majority of *E. coli* strains are classified as shigatoxigenic/verotoxigenic, enterotoxigenic, enteropathogenic, enterohaemorrhagic strains causing manifestation of many gastrointestinal disorders in livestock and human [21,22]. During the present investigation, the *E. coli* isolated from diarrhoeic and healthy pigs of the farm were found to be avirulent type based upon the result of PCR detection. Hence, it was presumed that *E. coli* was not responsible for diarrhea in the piglets under the study. Neonatal piglet diarrhoea is also commonly of viral origin, of which *Rotavirus* is mainly incriminated. In a study of prevalence of *Rotavirus* in North East India [23] reported upto 30% prevalence by RT-PCR, which

indicate the main role of *Rotavirus* in piglet diarrhoea in this region. In the present report, all the samples were negative for *Rotavirus* and hence ruled out as a possible cause of gastroenteritis.

According to the European Food Safety Authority, salmonellosis was the second most commonly reported zoonotic infection in the European Union with *S. enteritidis* as the most frequently isolated serovar [24]. Approximately 50% of *Salmonella* infections are caused by three *Salmonella* serovars, these are Typhimurium, Enteritidis and Newport. In another research from pigs in Northeastern India, ninety five isolates of *Salmonella* enteric were isolated and characterized to five serovars viz., *S. typhimurium*, *S. enteritidis*, *S. gallinarum*, *S. paratyphi B* and *S. bareilly* [25]. In another study, five *Salmonella* isolates reported as *S. typhimurium* were recovered from 20 diarrhoeic pigs in Assam, India [26]. In the present study, all the four salmonellae were Rough strains and were carrying resistance *bla*_{TEM} and *bla*_{CMY-2} gene. Hence based on cultural and molecular findings, it may be assumed that the piglets in the farm were suffering from acute salmonellosis and non-responsive to the drugs administered.

Serotyping is one of the most commonly used methods for subtyping of *Salmonella*, which enable the source attribution and reveal the source of outbreaks. Traditional approach of serotyping is done by slide agglutination using antisera aiming at two cell surface antigens: somatic (O) lipopolysaccharides (LPS) and flagellar (H). Some *Salmonella* strains have incomplete LPS structures, referred to as rough strains, or do not express H antigens, therefore serotyping by agglutination cannot be performed on these isolates [27]. Lipopolysaccharides (LPS) are characteristic components of the cell wall of Gram negative bacteria; they are not found in Gram positive bacteria. They are localized in the outer layer of the membrane and are, in noncapsulated strains, exposed on the cell surface. They contribute to the integrity of the outer membrane, and protect the cell against the action of bile salts and lipophilic antibiotics [28,5]. Amplification of *invA* gene now has been recognized as an international standard for detection of *Salmonella* [29]. This gene encodes a protein in the inner membrane of bacteria which is responsible for invasion to the epithelial cells of the host [30].

Resistance to antimicrobials has emerged as a greater problem in human and veterinary medicine, which were mainly due to the production of extended spectrum betalactams (ESBLs) which have been increasingly detected in food animals and animal products and have gained widespread attention [31]. Resistance to ampicillin, cefalexin and

enrofloxacin is alarming as these are commonly used in the region to treat gastroenteritis infection in livestock and poultry. Regular surveillance of emerging antimicrobial resistance among zoonotic food-borne pathogens is required to ensure safe public health.

Antimicrobial resistance among diarrhoeagenic bacteria, especially the production of ESBLs, has been rising worldwide since the early 1990s [32]. In the present study, *Salmonella* rough strains harboured ESBLs genes (*bla*_{TEM} and *bla*_{CMY-2}), which may be due to the wide use of extended-spectrum cephalosporins in the region, or it may be associated with the high mobilization of the encoding genes [33,34]. Detection of *Salmonella* strain with a combination of virulence and ESBL marker genes is suggestive of the nature of selective pressure that promotes combinations of virulent and resistant strains. The finding related to MDR is alarming since the resistance drugs of the present study, were used frequently and effectively in the past for treatment of gastroenteritis infection.

The factors contributing to the sudden disease outbreak might be due to introduction of organisms through introduction of infected animals from across border and also indicated towards poor hygienic and managerial practices prevailing in unorganised farms. Regular monitoring for *Salmonella* in food animals is very much necessary which must include adequate antimicrobial susceptibility testing and molecular characterization.

4. CONCLUSION

Salmonellosis^{TS} pathogenesis is highly complex and hence, its control programme is an enormous challenge to public health due to various serovars which may emerge and re-emerge in different geographical areas. Detection of *Salmonella* strains detected in the present study are of great zoonotic importance and a threat to public health as well as animal production due to the presence of ESBL resistance genes. Therefore, a comprehensive strategy involving regular surveillance at state and national level, preventive and proper treatment of the disease should be initiated in controlling the disease.

REFERENCES

1. Hur, J., Choi, Y.Y., Park, J.H., Jeon, B.W., Lee, H.S., Kim, A.R. and Lee, J.H. (2011) Antimicrobial resistance, virulence associated genes, and pulsed-field gel electrophoresis profiles of *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolated from piglets with diarrhea in Korea. *Can. J. Vet. Res.*, 75: 49-56.
2. Grimont, P.A.D. and Weill, F. (2007) *Antigenic Formulae of the Salmonella Serovars*. 9th ed. Paris: WHO Collaborating Center for Reference and Research on Salmonella Institute Pasteur.
3. Lan R, Reeves PR, Octavia S (2009) Population structure, origins and evolution of major *Salmonella enterica* clones. *Infect Genet Evol* 9: 996 -1005.

- 200 4. Agbaje M, Begum RH, Oyekunle MA, Ojo OE, Adenubi OT (2011) Evolution of Salmonella
201 nomenclature. *Folia Microbiol* 56: 497–503.
- 202 5. CDC, 2011. National Salmonella Surveillance Overview. Atlanta, Georgia: US Department of
203 Health and Human Services, Centers for Disease Control and Prevention. July ed; 6.
- 204 6. Zhao Y (2001) Virulence factors of Salmonella enterica serovar Enteritidis. Ph.D thesis, Submitted
205 to Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands
- 206 7. Mao Y, Zhu C, Boedeker EC (2003) Foodborne enteric infections. *Curr Opin Gastroenterol* 19: 11–
207 22
- 208 8. Pfeiffer ML, DuPont HL, Ochoa TJ (2012) The patient presenting with acute dysentery- a
209 systematic review. *J Infect* 64: 374–386
- 210 9. Ewing, W.H. 1986. Edward and Ewing's Identification of Enterobacteriaceae, 4th edn. New York
211 Elsevier, pp. 1-536.
- 212 10. World Health Organization (2009). Manual of rotavirus detection and characterization methods.
213 Geneva, Switzerland.
- 214 11. Gabbay, Y.B., Borges, A.A., Oliveria, D.S., Linhares, A.C., Mascarenhas, J.D., Barardi, C.R.,
215 Simoes, C.M., Wang, Y., Glass, R.I., Jiang, B. 2008. Evidence for zoonotic transmission of group C
216 rotaviruses among children in Belem, Brazil. *J. Medical Virol*, 80: 1666-1674.
- 217 12. Paton, J.C., Paton, A.W. 1998. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia
218 coli infections. *Clinical Microbiology Reviews*, 11: 450-479.
- 219 13. Phipps, S.S., Mecca, J.J., Weiss, J.B. 1995. Multiplex PCR Assay and Simple Preparation Method
220 for Stool Specimens Detect Enterotoxigenic Escherichia coli DNA during Course of Infection. *J Clin*
221 *Microbiol.*, 1054-1059.
- 222 14. Prager, R., Fruth, A., Tschape, H. (1995). Salmonella enterotoxin (stn) gene is prevalent among
223 strains of Salmonella enteric but not among Salmonella bongori and other enterobacteriaceae. *FEMS*
224 *Immunol. Med. Microbiol.*, 12: 47-50.
- 225 15. Galan, J.E., Ginocchio, C. and Costeas, P. 1992. Molecular and functional characterization of the
226 Salmonella gene invA: homology of invA to members of a new protein family. *J. Bacteriol.*, 174:
227 4338-4349.
- 228 16. Weill, F.X., Demartin, M., Laetitia Fabre, L. and Grimont Patrick, A.D. 2004. Extended-
229 spectrum-beta lactamase (TEM-52)-producing strains of Salmonella enterica of various serotypes
230 isolated in France. *J. Clin. Microbiol*, 42:3359-3362.
- 231 17. Schjorring, S., Struve, C., Krogfelt, K.A. 2008. Transfer of antimicrobial resistance plasmids from
232 Klebsiella pneumoniae to Escherichia coli in the mouse intestine. *J. Antimicrob. Chemother*, 62:
233 1086-1093.
- 234 18. Perez, F.J. and Hanson, N.D. 2002. Detection of Plasmid-Mediated AmpC β -Lactamase Genes in
235 Clinical Isolates by using Multiplex PCR. *J. Clin. Microbiol*, 40.6:2153-2162.
- 236 19. Akopyanz, N., Bukanov, N.O., Westblom, T.U., Kresovich, S. and Berg, D.E. 1992. DNA
237 diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting.
238 *Nucleic Acids. Res*, 20(19): 5137-5142.
- 239 20. Clinical Laboratory Standards Institute (2013). Performance standards for antimicrobial
240 susceptibility testing. Twenty third informational supplement. M100-S23. Wayne, USA.
- 241 21. Kim, Y.J; Kim, J.I; Hur, J and Lee, J.H. (2010) Isolation of Escherichia coli from piglets in South
242 Korea with diarrhea and characteristics of the virulence genes. *Can. J. of Vet. Res*; 74: 59-64.
- 243 22. Vu-Khac, H., Holoda, E., Pilipcinec, E., Blanco, M., Blanco, J.E., Mora, A., Dahbi, G., López, C.,
244 González, E.A., Blanco, J. (2006). Serotypes, virulence genes, and PFGE profiles of Escherichia coli
245 isolated from pigs with postweaning diarrhoea in Slovakia. *BMC Vet. Res.*, 2: 10.

23. Dubal, Z.B., Bhilegaonkar, K.N., Barbuddhe, S.B., Kolhe, R.P., Kaur, S., Rawat, S., Nambiar, P., Karunakaran, M. (2013). Prevalence and genotypic (G and P) determination of porcine group A rotaviruses from different regions of India. *Trop. Anim. Health*, 45: 609–615.
24. Westrell, T; Ciampal, N, Boelaert, F; Helwigh, B; Korsgaard, H; Chriel, M; Ammon, A. and Makela, P (2009) Zoonotic infections in Europe in 2007: a summary of the EFSA-ECDC annual report. *Eurosurveillance*; Vol. 14: 3; 1-2.
25. Murugkar, H.V., Rahman, H., Kumar, A. and Bhattacharya, D. 2005. Isolation, phage typing and antibiogram of *Salmonella* from man and animals in north eastern India. *Ind. J. Med. Res*, 122:237-242.
26. Borah, P.P., Saikia, G.K., Sharma, R.K., Baishya, N. (2013). Characterization of *Salmonella* isolates recovered from animals and man. *The North East. Vet.*, XIII(2): 21-24.
27. Löfström, C, Boel, J, Sisic, E, Thomsen, M, Sørensen, G (2013). Molecular serotyping of rough *Salmonella* strains isolated from Danish pork production. 10th International Meeting on Microbial Epidemiological Markers, Paris, France.
28. Mayer, H. et al., (1985) Analysis of Lipopolysaccharides of Gram-Negative Bacteria. *Methods in Microbiology* 18, 157-207.
29. Malorny B, Hoorfar J, Bunge C, Helmuth R (2003) Multicenter validation of the analytical accuracy of *Salmonella* PCR towards an international standard. *Appl Environ Microbiol* 69: 290-296.
30. Darwin KH and Miller VL (2009). Molecular basis of the interaction of *Salmonella* with intestinal mucosa. *Clin. Microbiol. Rev.*, 12: 405-428.
31. Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F., Butaye, P. (2010). BROADSPECTRUM beta-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. *FEMS Microbiol. Rev.*, 34: 295-316.
32. Buvens G, Bogaerts P, Glupczynski Y, Lauwers S, Pierard D. 2010. Antimicrobial resistance testing of verocytotoxin-producing *Escherichia coli* and first description of TEM-52 extended spectrum-lactamase in serogroup O26. *Antimicrob. Agents Chemother.* 54:4907-4909.
33. Barguigua A, Fatima EO, Mustapha T, Fatna B, Fatima H, Khalid Z, Mohammed T. 2011. Characterization of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from community in Morocco. *J. Med. Microbiol.* 60:1344-1352.
34. Lin Y, Lu M, Hui-Ling TH, Liu H, Chen C, Liu K, Lin C, Chiou C, Chiang M, Chen C, Yi-Chyi LY. 2011. Assessment of hypermucoviscosity as a virulence factor for experimental *Klebsiella pneumoniae* infections: comparative virulence analysis with hypermucoviscosity-negative strain. *BMC Microbiol.* 11:50.

Table-1. PCR amplification of target genes

Target gene	Sequence (5' – 3')	Annealing	Amplicon size (bp)	Reference
<i>stn</i>	(F) TTGTGTCGCTATCACTGGCAAC (R) ATTCGTAACCCGCTCTCGTCC	60 ⁰ C, 1m	617	Prager <i>et al.</i> (1995)
<i>invA</i>	(F) ACCACGCTCTTTTCGTCTGG (R) GAACTGACTACGTAGACGCTC	61 ⁰ C, 1m	941	Galan <i>et al.</i> (1992)
<i>eae</i>	(F) GACCCGGCACAAGCATAAGC (R) CCACCTGCAGCAACAAGAGG	61 ⁰ C, 45s	384	Paton and Paton(1998)
<i>stx1</i>	(F) ATAAATCGCCATTCGTTGACTAC (R) AGAACGCCCACTGAGATCATC	61 ⁰ C, 45s	180	Paton and Paton(1998)
<i>stx2</i>	(F) GGCACGTCTGAACTGCTCC (R) TCGCCAGTTATCTGACATTCTG	61 ⁰ C, 45s	255	Paton and Paton(1998)
<i>hlyA</i>	(F) GCATCATCAAGCGTACGTTCC (R) AATGAGCCAAGCTGGTTAGCT	61 ⁰ C, 45s	534	Paton and Paton(1998)
<i>LT</i>	(F) GCGGACAGATTATACCGTGC (R) CGGTCTCTATATTCCTGTT	50 ⁰ C, 45s	450	Phipps <i>et al.</i> (1995)
<i>ST</i>	(F) ATTTTMTTTCTGTATRTCTT (R) CACCCGGTACARGCAGGATT	50 ⁰ C, 45s	190	Phipps <i>et al.</i> (1995)
VP7 RotaA	TTGACTAARGGRTGGCCAACWGG TCGCATCATHCKYTCNGTTTGTGG	56 ⁰ C, 45s	540	Designed primers
VP6 RotaC	1.CTCGATGCTACTACAGAATCAG 2.AGCCACATAGTTCACATTTTCATCC	46 ⁰ C, 30s	356	Gabbay <i>et al.</i> (2008)
<i>bla</i> _{TEM}	(F) ATAAATTCCTTGAAGACGAAA (R) GACAGTTACCAATGCTTAATC	53 ⁰ C, 30s	1080	Weill <i>et al.</i> , 2004
<i>bla</i> _{SHV}	(F) CGCCTGTGTATTAATCAGTGAGGCAC (R) TTGCCAGTGCTCGATCAGCG	60 ⁰ C, 30s	842	Schjorring <i>et al.</i> , 2008
<i>bla</i> _{CMY-2}	(F) TGGCCAAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	60 ⁰ C, 45s	462	Perez and Hanson, 2002
<i>bla</i> _{CTX-M}	(F) CAATGTGCAGCACCAGTAA (R) CGCGATATCGTTGGTGGTG	58 ⁰ C, 45s	540	Perez and Hanson, 2002

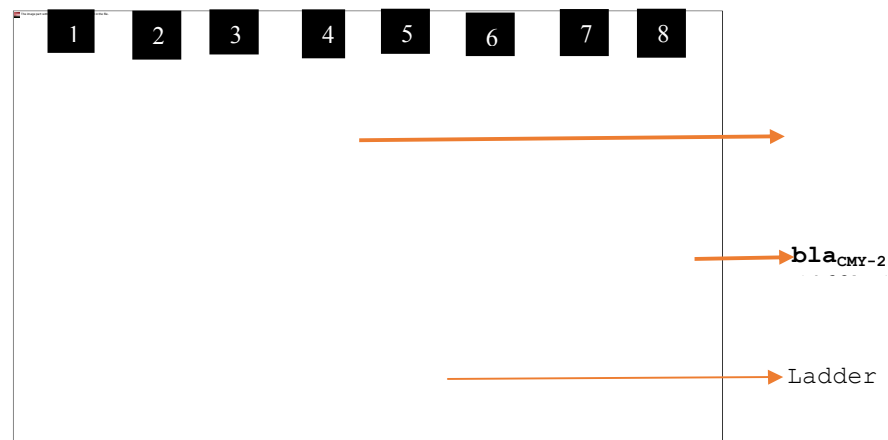


Figure 1. Agarose gel electrophoresis showing the PCR amplicons of *Salmonella* rough strains

318 Lane 1, 3, 4: Positive field isolates (1080bp, bla_{TEM});
319 Lane 7 and 8: Positive field isolates (462bp, bla_{CMY-2})
320 Lane 2 and 6: No template control;
321 Lane 5: 100 bp DNA ladder