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Review Article

LIVESTOCK AS A RESERVOIR OF EXTENDED SPECTRUM β-LACTAMASE PRODUCING GRAM-NEGATIVE ORGANISMS

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Resistance to β-lactam antibiotics in gram negative organisms (GNOs) is mediated through different mechanisms. Mutations in the penicillin binding proteins, disruption of porin proteins and efflux systems, production of enzyme that hydrolyzes and inactivates the β-lactam ring of the drug are some of them. Extended-spectrum β-lactamases (ESBLs) are defined as plasmid-encoded enzymes found in the Enterobacteriaceae, frequently in *Escherichia coli* and *Klebsiella pneumoniae*, that confer resistance to a variety of â-lactam antibiotics, including penicillins, 2nd, 3rd and 4th-generation cephalosporins and monobactams, but usually not the carbapenems or the cephamycins. ESBLs are classified mainly by two approaches: functional classification that are based on functional properties of enzymes and molecular classification based on proteins sequence similarity of the enzyme. For detection of ESBLs from samples collected from community and hospital settings, both phenotypic and genotypic approaches are needed. The phenotypic tests would only confirm a preliminary suspect for presence of ESBLs. For more detail and genetic evaluation of a sample, genotypic tests are necessary. Various livestock species are observed to be the reservoir of ESBL producing GNOs among which poultry and pigs are quite imminent. The present review narrates some of the recent findings on the subject in global, national and regional settings.

Key words : Antibiotic resistance, CTX-M, ESBL, *Escherichia coli*, *Klebsiella pneumoniae*, SHV, TEM.

Evolutions of antibiotics probably date back a million of years ago as an outcome of struggle for survival of microorganisms in environment although the scientific world came to know its existence only after 1945 when Sir Alexander Fleming was recognized with Nobel prize (Medeiros, 1997). Unknowingly human civilization is using antibiotics since 350-550 CE as detectable amount of tetracycline have been found in the fossils of human bone from Sudanese Nubia which belonged to that period (Bassett *et al.*, 1980; Aminov, 2010). The therapeutic use of antibiotics began with Paul Ehrlich's concept of 'magic bullet' targeting pathogens only without causing any damage to the host.

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This idea led Ehrlich and his colleagues to develop a synthetic antibacterial compound, arsphenamine in 1909, but it had many side effects (Aminov, 2010). Consequently, Prontosil, a sulfonamide, discovered by Gerhard Domagk (1932), became the first and the most accepted antibiotic (Sneader, 2005). Nonetheless, in 1928 Alexander Fleming was the first to report antibacterial property of Penicillium (Fleming, 1929). Almost after 12 years, Howard Florey and Ernest Chain successfully extracted the penicillin molecule (Chain *et al*., 2005). Penicillin soon became widely used as cure for infection in hospitals caused by Gram-positive bacteria (Medeiros, 1997). On account of extensive use of penicillin and evolutionary selective pressure antibiotic resistant bacteria began to proliferate (Essack, 2001). *Staphylococcus aureus* resistance to penicillin was reported in hospitals in England shortly within the same year of its introduction (Barber, 1948).

Penicillin is classified as β-lactam antibiotic which inhibits the synthesis of cell wall that leads to bacterial death (Essack, 2001). Resistance to β-lactam antibiotics in gram negative organisms (GNOs) is mediated through different mechanisms such as mutations in the penicillin binding proteins (PBPs, binding site of the drug), disruption of porin proteins and efflux systems which reduces the cell wall permeability and moreover, production of enzyme that hydrolyzes and inactivates the β-lactam ring of the drug. There are more than 1000 β-lactamase enzymes that can be grouped into four main classes (A–D) based upon amino acid sequence homology (Bush and Jacoby, 2009). The most clinically important class A enzymes are known as extended-spectrum β-lactamases (ESBLs). Currently ESBLs are defined as plasmid-encoded enzymes found in the Enterobacteriaceae, frequently in *Escherichia coli* and *Klebsiella pneumoniae*, that confer resistance to a variety of ß-lactam antibiotics, including penicillins, 2nd, 3rd and 4th-generation cephalosporins and monobactams (e.g. aztreonam), but usually not the carbapenems or the cephamycins (e.g. cefoxitin) (EFSA Panel on Biological Hazards, 2011). Although, species like Haemophilus influenzae (Pasteurellaceae), *Pseudomonas aeruginosa* (Pseudomonadaceae) and *Neisseria gonorrhoeae* (Neisseriaceae) are also known as ESBL producer (Rahman, 2014).

CLASSIFICATION

ESBLs are classified mainly by two approaches: functional classification by Bush, Jacoby and Medeiros based on functional properties of enzymes and molecular classification by Ambler based on proteins sequence similarity of the enzyme (Ambler, 1980; Bush *et al*., 1995). According to the enzymes produced, ESBLs are categorized into three groups i.e. TEM (except TEM-1), SHV (except SHV-1 and 2) and CTX-M (EFSA Panel on Biological Hazards, 2011). Among them, CTX-M is observed as the most prevalent type throughout the world (Carattoli, 2013). While TEM and SHV-type ESBLs arise via substitutions in strategically positioned amino acids from the natural narrow spectrum TEM-1/-2, or SHV-1-â-lactamase, all CTX-M enzymes

demonstrate an ESBL phenotype (Gniadkowski, 2008). For example, SHV-2-â-lactamase arises from SHV-1-â-lactamase by replacement of glycine with serine at the 238 position. This mutation alone accounts for the extended-spectrum properties of this â-lactamase. Similarly TEM-type ESBLs derives from TEM-1 and TEM-2. TEM-1 was first reported in 1965 from an *Escherichia coli* isolate from a patient in Greece, named Temoneira (hence the designation TEM) (Datta and Kontomichalou, 1965). However, there are other enzymes as well that are known to produce by ESBL producing GNOs (e.g. PER, GES, VEB-1, BES-1, CME-1, FEC-1 and SFO-1) (Bradford, 2001; Shaikh *et al*., 2015).

DETECTION

ESBL producing GNOs lead to failure in treatment with major cephalosporin drugs like ceftazidime, ceftriaxone, cefotaxime and thus becoming a major concern worldwide. Throughout the last 2 decades many modern â-lactam antibiotics were developed to overcome treatment failure but with each new antibiotic, resistance strain has emerged. Most likely it is because of extensive use of antibiotic and associated selective pressure (Bradford, 2001).

There are standard guidelines for phenotypic detection published by the United States Clinical and Laboratory Standards Institute (CLSI) and the United Kingdom Health Protection Agency (HPA) for detection of ESBL in Enterobacteriaceae (*E. coli, Klebsiella, Proteus*). HPA also includes guideline for other species such as *Salmonella*. The CLSI and HPA published methods show high sensitivity of up to 94% and specificity of 98% for detecting ESBLs in Enterobacteriaceae (Pitout and Laupland, 2008). CLSI suggested a two-step method, first is examination of zone of inhibition by using a cephalosporin (cefotaxime, ceftriaxone, ceftazidime, cefpodoxime and aztreonam), followed by zone examination by using combination of a cephalosporin and a â-lactam inhibitor (clavulanate). A $\geq$ 5mm increase in zone diameter for cephalosporin/ clavulanate combination versus cephalosporin zone when tested alone indicates probable ESBL production.

Some common methods used in clinical laboratories are double disk- synergy test (DDST), ESBL E-strip, combination disk method and three dimensional method. Automated test such as VITEK-2 ESBL test and Phoenix ESBL tests are also available. Every method has its own pros and cons, e.g. DDST is easy to use but optimal sensitivity cannot be guaranteed because distance for antibiotic disk placement is not standardized. ESBL E- Stripe is easy to use but result interpretation is challenging and it is not as sensitive as DDST. VITEK-2 is designed for detection of ESBL within 2-15 minutes but it fails to differentiate between ESBLs and AmpC (Rahman *et al*., 2014). None of these tests are 100% specific for accurate detection of ESBL phenotypes.

Phenotypic tests detect the presence of ESBL merely, but to identify the type of ESBL enzymes (TEM, SHV or CTX-M) genotypic test is needed. Point mutation

around the active sites of parent enzymes sequence (TEM-1, TEM-2 and SHV-1) has extended its activity spectrum and thus led to the evolution of other derivatives. Therefore, identification of an isolate whether it is related to TEM or SHV is a difficult task. Earlier molecular detection was done by determining isoelectric point but with more than 90 types of TEM â-lactamase and many of them having similar isoelectric point, it is no longer possible to determine ESBLs (Bradford, 2001). There are other techniques available as well e.g. DNA probe, polymerase chain reaction (PCR), ligase chain reaction (LCR) and nucleotide sequencing. Some PCR based techniques do not use sequencing for ESBLs detection e.g. PCR with restriction fragment length polymorphism (PCR-RFLP) and PCR with single-strand conformational polymorphism (PCR-SSCP). The most used and acceptable method for molecular detection of ESBLs is PCR amplification followed by nucleotide sequencing. Point mutation in ESBL genes like bla_{TEM} or bla_{SHV} could easily be identified with this method.

Therefore, for detection of ESBLs from samples collected from community and hospital settings both phenotypic and genotypic approaches are needed. The phenotypic tests would only confirm a preliminary suspect for presence of ESBLs. For more details and genetic evaluation of a sample genotypic tests are necessary.

LIVESTOCK AS A RESERVOIR OF ESBL PRODUCING GNOs

First human infection with ESBL producing *Klebsiella pneumoniae* was reported from Germany in 1983 (Knothe *et al*., 1983). After 15 years, cattle and pigs were detected as reservoirs of *Salmonella* with CMY-2 in United States (Fey *et al*., 2000; Winokur *et al*., 2000). The poultry as a reservoir of CMY-2, CTX-M-14 and SHV-12-producing *E. coli* was first detected in Spain (Brinas *et al*., 2003). The study conducted by European Food Safety Authority (EFSA) observed that the overall prevalence of ESBL-producing *E. coli* isolates in cattle in the European Union was 1.6%, with a range of 0% (Austria, Denmark, Finland, Sweden) to 6.5% (Hungary) (EFSA, 2011). In United States, the prevalence of ESBL-producing *E. coli* and *Salmonella* in cattle was 6% and 2.4%-14.5%, respectively (Wittum *et al*., 2010 and USDA, 2011). In Asian cattle, the prevalence of ESBL-producing *E. coli* is 1-33% (Asai *et al*., 2011; Hiroi *et al*., 2011; Ho *et al*., 2011 and Zheng *et al*., 2012).

Poultry is the most important reservoir of ESBL-producing GNOs as numerous reports concern it. In European Union, mean prevalence of ESBL-producing *E. coli* was 8.5%, with a range of 0% (Denmark) to 26.4% (Spain) (Frye and Fedorka-Cray, 2007). In Asia, prevalence of ESBL-producing *E. coli* in poultry ranges between 8 and 60% (Asai *et al*., 2011; Hiroi *et al*., 2011, 2012; Ho *et al*., 2011; Li *et al*., 2010 and Zheng *et al*., 2012).

Not much information on ESBL producing *E. coli* in livestocks is available in India. In one earlier study negative correlation in possession of ESBL genes and shiga-toxin genes in *E. coli* isolated from healthy buffalo

was revealed (Mahanti *et al.*, 2013). However, positive correlation between ESBL genes and other virulence genes (shiga toxins) was observed in *E. coli* isolated from diarrhoeic piglets in Mizoram state (Mandakini *et al.*, 2015). In West Bengal state, 29% prevalence of ESBL-producing *E. coli* was detected in broiler birds (Samanta *et al.*, 2015), whereas, the studied backyard poultry (RIR breed) were free from carriage of ESBL-producing *E. coli* and *Salmonella* (Samanta *et al.*, 2014a; 2014b). Probably lack of antibiotic use in backyard birds is the reason behind the finding. In Kuroiler birds, 45% of *E. coli* 23.9% of *Klebsiella* isolates were found to harbour any of the major ESBL genes studied (bla_{TEM}, bla_{SHV}, bla_{CTX-M}) (Ghosh, 2015). Majority of the ESBL gene-possessing isolates (25 *E. coli* and 16 *Klebsiella*) from Kuroiler birds possessed bla_{SHV} gene whereas, majority of the ESBL-*E. coli* isolates (43%) from broilers possessed $bla_{CTX-M.}$ ESBL-producing *E. coli* was also detected to be associated with methicillin-resistant *Staphylococcus epidermidis* (MRSE) and methicillin-resistant *Staphylococcus aureus* (MRSA) causing clinical and subclinical mastitis in Holstein Friesian crossbred and non-descript cows in West Bengal (Bandyopadhyay *et al.*, 2015).

It may be concluded that the knowledge of Indian livestock as ESBL-GNO reservoir is insufficient that needs detailed studies in future. It is important not only from the point of animal health issues but also epidemiological perspective as the occupational workers closely associated with livestock might be responsible for transmission of resistant GNOs into the community.

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REFERENCES

Ambler RP, 1980. The structure of â-lactamases. Philos Trans R Soc Lond B Biol Sci, 289: 321-331

Aminov R, 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbio, 1

Asai T, Masani K, Sato C, Hiki M, Usui M *et al.*, 2011. Phylogenetic groups and cephalosporin resistance genes of *Escherichia coli* from diseased food-producing animals in Japan. Acta Vet Scand, 53 : 52

Bandyopadhyay S, Samanta I, Bhattacharyya D, Nanda PK, Kar D, *et al*., 2015. Co-infection of methicillin-resistant *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* and extended spectrum â-lactamase producing *Escherichia coli* in bovine mastitis-three cases reported from India. Vet Q, 35(1): 56-61

Barber M, 1948. Infection by penicillin resistant Staphylococci. Lancet, 2: 641-644

Bassett EJ, Keith MS, Armelagos GJ, Martin DL and Villanueva AR, 1980. Tetracycline-labelled human bone from ancient Sudanese Nubia (A.D. 350). Science, 209: 1532-1534

Bradford P, 2001. Extended-Spectrum â-Lactamases in the 21st Century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev, 14(4) : 933-951

Brinas L, Moreno MA, Zarazaga M, Porrero C, Saenz Y *et al*., 2003. Detection of CMY-2, CTX-M-14, and SHV-12-â-lactamases in *Escherichia coli* fecal sample isolates from healthy chickens. Antimicrob Agents Chemother, 47: 2056-2058

Bush K and Jacoby GA, 2009. Updated functional classification of â-lactamases. Antimicrob Agents Chemother, 54: 969-976

Bush K, Jacoby GA and Medeiros AA, 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother, 39: 1211-1233

Carattoli A, 2013. Plasmids and the spread of resistance. Int J Med Microbiol, 303: 298-304

Chain E, Florey H, Gardner A, Heatley N, Jennings M, Orr-Ewing J, Sanders A and Peltier L, 2005. THE CLASSIC: Penicillin as a Chemotherapeutic Agent. Clin Ortho Related Res, 439: 23-26

Datta N and Kontomichalou P, 1965. Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. Nature, 208(5007) : 239-241

EFSA (European Food Safety Authority), 2011. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum â-lactamases and/or AmpC â-lactamases in food and food-producing animals. EFSA J, 9: 2322

Essack S, 2001. The development of beta-lactam antibiotics in response to the evolution of beta-lactamases. Pharma Res, 18(10): 1391-1399

Fey PD, Safranek TJ, Rupp ME, Dunne EF, Ribot E *et al*., 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. New England J Med, 342: 1242-1249

Fleming A, 1929. On the antibacterial action of cultures of a Penicillium with special reference to their use in the isolation of b. influenzæ. Br J Exp Pathol, 10(3): 226-236

Frye JG and Fedorka-Cray PJ, 2007. Prevalence, distribution and characterization of ceftiofur resistance in *Salmonella enterica* isolated from animals in the USA from 1999 to 2003. Int J Antimicrob Agents, 30 : 134-142

Ghosh P, 2015. Isolation and characterization of *Klebsiella* and *Escherichia coli* from Kuroiler and indigenous birds. M.V.Sc. thesis submitted to West Bengal University of Animal and Fishery Sciences, Kolkata

Gniadkowski M, 2008. Evolution of extended-spectrum â-lactamases by mutation. Clin Microbiol Infect, 14 (1): 11-32

Hiroi M, Harada T, Kawamori F, Takahashi N, Kanda T, *et al.*, 2011. A survey of â-lactamase-producing *Escherichia coli* in farm animals and raw retail meat in Shizuoka Prefecture, Japan. Japanese J Infect Dis, 64: 153-155

Hiroi M, Yamazaki F, Harada T, Takahashi N, Iida N *et al*., 2012. Prevalence of extended-spectrum â-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in food-producing animals. J Vet Med Sci, 74: 189-195

Ho PL, Chow KH, Lai EL, Lo WU, Yeung MK, Chan J, Chan PY and Yuen KY, 2011. Extensive dissemination of CTX-M-producing *Escherichia coli* with multidrug resistance to 'critically important' antibiotics among food animals in Hong Kong, 2008–2010. J Antimicrob Chemother, 66: 765-768

Knothe H, Shah P, Krcmery V, Antal M and Mitsuhashi S, 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infect, 11 : 315-317

Li J, Ma Y, Hu C, Jin S, Zhang Q *et al*., 2010. Dissemination of cefotaxime-M-producing *Escherichia coli* isolates in poultry farms, but not swine farms, in China. Foodborne Pathog Dis, 7: 1387-1392

Mahanti A, Samanta I, Bandopaddhay S, Joardar SN, Dutta TK *et al*., 2013. Isolation, molecular characterization and antibiotic resistance of Shiga Toxin-Producing *Escherichia coli* (STEC) from buffalo in India. Lett Appl Microbiol, 56(4): 291-298

Mandakini R, Dutta TK, Chingtham S, Roychoudhury P, Samanta I *et al*., 2015. ESBL-producing Shiga-toxigenic *E. coli* (STEC) diarrhoea in associated with piglet India. Trop Anim Health Prod, 47(2) : 377-381

Medeiros A, 1997. Evolution and dissemination of â-Lactamases accele-rated by generations of â-Lactam Antibiotics. Clin Infect Dis, 24(1) : S19-S45

Pitout J and Laupland K, 2008. Extended-spectrum â-lactamase-producing Enterobacteriaceae : an emerging public-health concern. Lancet Infect Dis, 8(3) : 159-166

Rahman M, Rahman M and Jahan W, 2014. Clinical laboratory and molecular detection of extended spectrum beta lactamases: a review update. Bangladesh J Infect Dis, 1(1): 12

Samanta I, Joardar SN, Das PK and Sar TK, 2015. Comparative possession of shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase genes in *E. coli* isolated from backyard and farmed poultry in West Bengal, India. Iranian J Vet Res, 16(1): 90-93

Samanta I, Joardar SN, Das PK, Das P, Sar TK *et al*., 2014a. Virulence repertoire, characterization and antibiotic resistance pattern analysis of *Escherichia coli* isolated from Backyard Layers and their environment in India. Avian Dis, 58 (1) : 39-45

Samanta I, Joardar SN, Das PK, Sar TK, Bandopadhaya S *et al*., 2014b. Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. J Appl Poult Res, 23 (3): 536-545

Shaikh S, Fatima J, Shakil S, Rizvi S and Kamal M, 2015. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Bio Sci, 22(1): 90-101

Sneader W, 2005. Drug Discovery (The History). Van Nostrand's Scientific Encyclopedia

USDA (US Department of Agriculture), 2011. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Animal Arm Annual Report, 2009.http://www.ars.usda.gov

Winokur PL, Brueggemann A, DeSalvo DL, Hoffmann L, Apley MD *et al*., 2000. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC-â-lactamase. Antimicrob Agents Chemother, 44: 2777-2783

Wittum TE, Mollenkopf DF, Daniels JB, Parkinson AE, Mathews JL *et al*., 2010. CTX-M-type extended-spectrum â-lactamases present in *Escherichia coli* from the feces of cattmle in Ohio, United States. Foodborne Pathog Dis, 7: 1575-1579

Zheng H, Zeng Z, Chen S, Liu Y, Yao Q *et al*., 2012. Prevalence and characterization of CTX-M â-lactamases amongst *Escherichia coli* isolates from healthy food animals in China. Int J Antimicrob Agents, 39: 305-310

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