AMR at human animal interface: PGIMER research updates

Dr. Neelam Taneja
Professor and In-charge Enteric Laboratory
Postgraduate Institute of Medical Education and Research
Chandigarh

8.2017

PGIMER

2000 bed tertiary care referral centre in Chandigarh, North India

Caters to patients from seven adjoining States (Chandigarh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, western parts of Uttar Pradesh, Uttaranchal, and some parts of Rajasthan) thus representing a large geographical area

2,42,3501 outpatients and 87973 admission in 2016

8.2017

Enteric Lab

Diagnostic(conventional as well as molecular)

- Urine and Stool samples
- Load- 45000 /year
- NABL accreditation

Surveillance and referral services investigating and managi
outbreaks of cholera, and food poisoning in this geographic area

Research in the field of diarrhea , food borne infections a
urinary tract infections with special focus on epidemiology a
drug resistance and pathogenesis

Antimicrobial resistance in Enteric and Uro pathogens is bei
studied at community and hospital level by both phenotypic a
molecular assays

Conventional Culture for Salmonella, Shigella ,Vibrio cholerae ,Campylobacter ,STEC and Yersinia,C.difficle

Antigen detection for STEC, Campylobacter,C difficle

Molecular tests

- Multiplex PCR for diarrhoeagenic *E.coli*
- *Shigella* PCR
- STEC PCR
- Campylobacter PCR

Rapid dipstick for cholera and dysentery

Food borne Pathogens

Vibrios, Bacillus cereus, Shigella ,Aeromonas, Pleisomonas, Yersinia Campylobacter, *Staphyloccocus aureus*, Listeria, testing of milk samples by total plate counts, methylene blue test , turbidity test , coliform tests fo milk, full water testing for coliforms, *E coli*, Enterococci, *V. cholerae* testing of water directly for diarrhoeagenic *E.coli* using in house protocols (PCR).

Molecular assays to detect directly from food samples

Special food (RUTF) which was developed to treat malnutrition was tested by us for component analysis and shelf life of the prepared food

Receive request from Government labs for testing commercial food and water samples

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Capacity for molecular epidemiology

- AFLP
- PFGE
- MLVA
- Ribotyping
- Plasmid typing
- Rep-PCR and RAPD
- MLST
- WGS

Public Health Contribution

blic health role and surveillance

Investigated 25 outbreaks of cholera and gastroenteritis.

Visit the area and collect water samples, stool samples and study the factors responsible cholera.

Due to fast identification of source of water contamination, we could control the chol outbreaks in a week .

We are also studying the molecular epidemiology of *V. cholerae* causing the rec epidemics in our region byAFLP, ribotyping and PFGE.

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WHO APW project and DFC

rengthening district public health laboratories for laboratory surveillance of communicable diseases of tbreak potential
m of phase 1

To assist in capacity building of the district laboratories.

- Punjab-Moga, Bhatinda, Hoshiarpur,Sangrur
- Haryana-Ambala, Faridabad, Bhiwani, Panchkula
- Uttarakhand-Dehradun, Haridwar, Tehri Garhwal,Pauri

To assist in technical operations & provision of resources needed to ensure required quality of laboratory procedures.

To formulate a framework for establishment of quality control & quality assessment schemes in these laboratories.

To periodically visit the laboratories at district level & monitor the progress

WHO APW project and DFC-Phase 2

Stool referral system :A total of 336 stool samples were collected and submitted from the following centers- Moga (N=58 , Ambala (N=38), Sangrur (N=17), Panchkula (N=72) and Pauri (N=32) DPHLs. These samples were collected from PHC an DPHL level by active surveillance

Most of the samples (231, 68%) were sent by local couriers and 95% were received in proper conditions, though some were not sent in double packaging

Outbreaks of cholera were reported from Ambala, Moga and Panchkula ,samples were also submitted from suspected cases from Sangrur , Uttarakhand and Chandigarh for confirmation .

Fourteen outbreaks of diarrhoea / food poisoning occurred in 2010 and 2011 followed by 4 outbreaks each of dengue chickenpox, measles and viral fever , 3 of jaundice , 2 of typhoid one each of viral encephalitis and rubella .

Tool for assessment of biosafety in laboratory

The culture facilities were established at the following centres- Ambala, Bhiwani, Haridwar , Pauri.

Quality assurance panels were sent and responded

Food borne illnesses

Foodborne illness are of serious concern to public health worldwide

90% bacterial infections followed by parasitic and viral

Major bacterial pathogens : *Campylobacter, Salmonella,* Diarrhoeagenic *coli, Listeria, Yersinia, Shigella, Vibrio*, etc.

Salmonella is the leading cause of death followed by *Campylobacter* (C Alert;2008)

Both occur in intestinal tract of sheep, goat, pigs and poultry

E. coli is commensal microbiota but some may be pathogenic strain
i.e. **EPEC, ETEC**, EAEC, EIEC, STEC

Transfer of virulence genes can occur through lateral gene transfer

Diarrhoeagenic *E. coli* caused maximum hospitalizations

Contaminated food and water are main sources of infections

Indian scenario

Burden of foodborne diseases is unknown

No surveillance system

No national database of epidemiology of common food borne pathogens

Foodborne infections occur as sporadic cases and also as outbreaks

Underreporting of foodborne outbreaks and cause is rarely established. Foodborne gastroenteritis is clubbed with acute diarrhoea and is not notifiable

In our region too foodborne illnesses are very common but no investigated or reported

In a study conducted by us, in collaboration with WHO, we found tha every year 1,400 to 31,000 cases of suspected food- and-water-borne infections were being reported at the district public health lab (DPHLs) across Punjab, Haryana and Uttarakhand

Every year one to three outbreaks of food poisoning reportedly occurred at the DPHLs (Unpublished data PGIMER) Almost all of these go uninvestigated

How antibiotics in livestock affect us

used for prophylactic, therapeutic and growth promotion

suppress gut flora leaving more nutrients to be absorbed by animal leading t greater gain in weight

Most of the antibiotic classes are the ones used for humans

Overuse has lead to emergence of antibiotic resistance

Residues of these antibiotics remain active in animals for certain time and ar also excreted in faeces

Consumption of trace levels of these residues in foods may alter human intestina microflora and cause resistance gene transfer

Low levels of abs released in environment accumulate and affect bacteria an cause transfer of resistance genes

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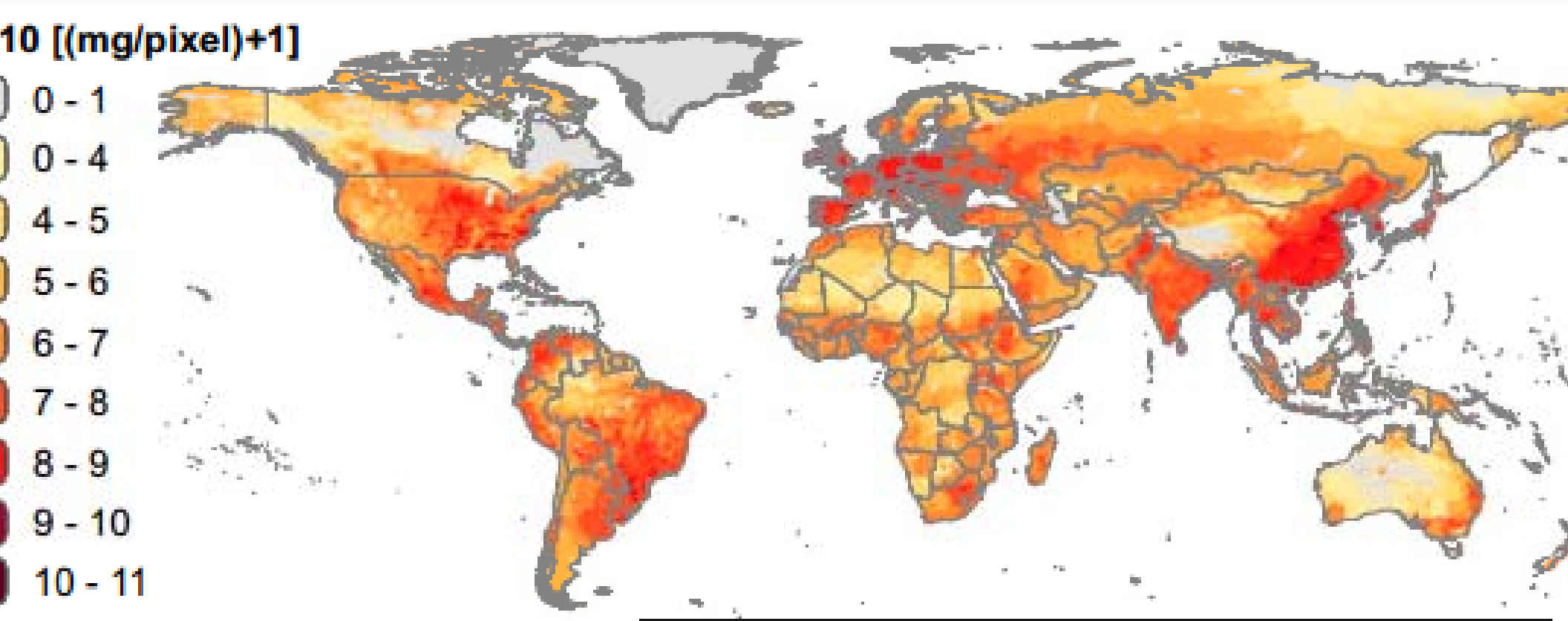
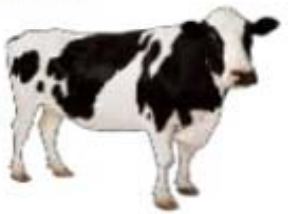


Global antimicrobial consumption in livestock (Van Bockerel; 2015)

Indian Livestock population: 2011(in Million Heads



Cattle
210

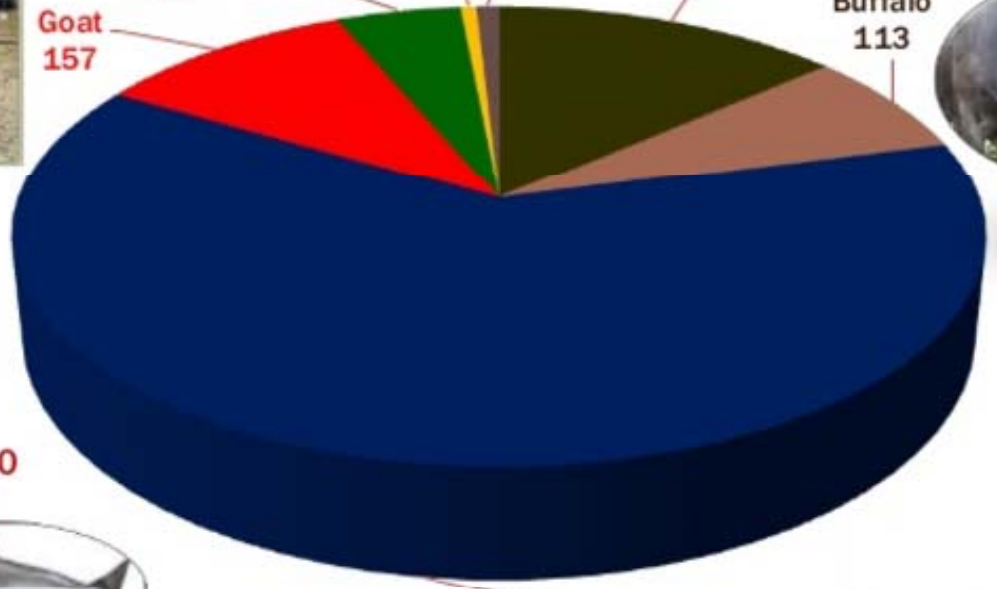
Pigs
9.5

Pets
14

Sheep
75

Goat
157

Buffalo
113



Aqua-80,000
Hectares

Poultry
968

K.S.Niranjan

Source: FAOSTAT

What we lack in India

Guidelines on antibiotic use in feed are available, but not implemente

Antibiotics used for human disease treatment are used in growt promoters

No monitoring of residue limits in food meat in Indian markets

We do not know the antibiotic resistance pattern or resistance ger pool in food animals

We are unaware of the rate of transmission of antibiotic resistance humans

lly imp
otics
istin
ofloxacin
omycin
floxacin
ikacin
oxcillin

ghly imp
tibiotics
tetracycline

pro-
acycline

ycycline

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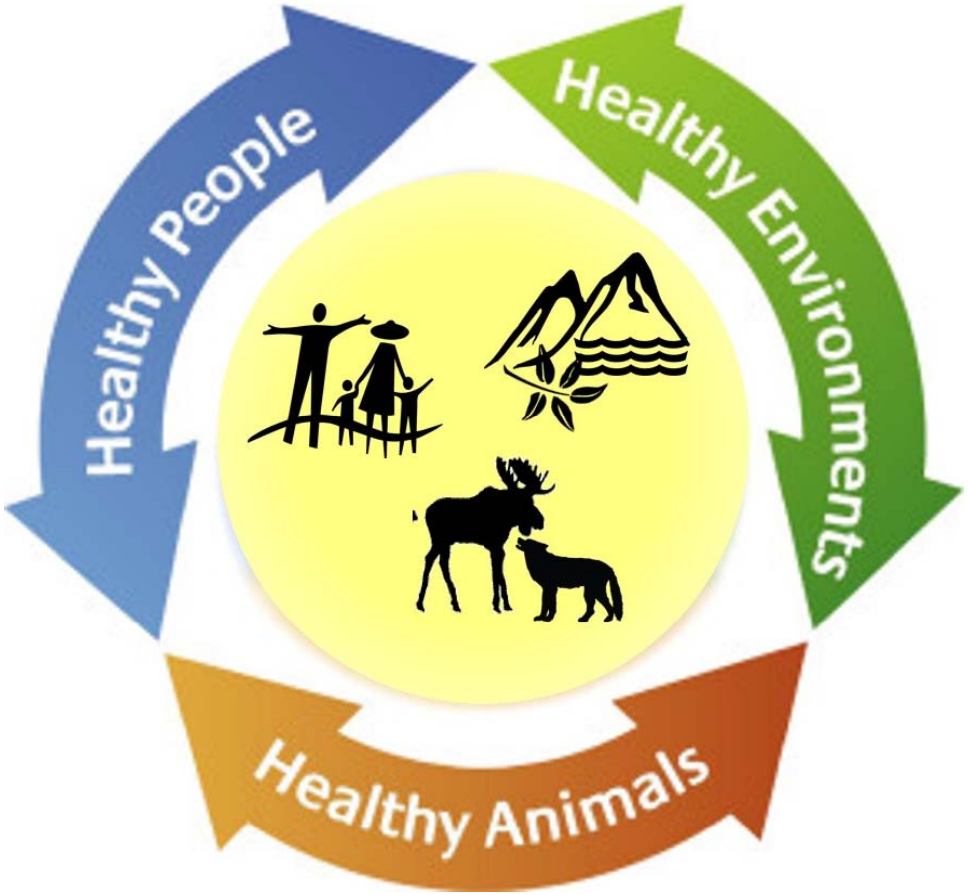
Antibiotics	Class / active compound	
Furazolidone	Nitrofuran	Human + veterinary
Bacitracin	Peptides from B. licheniformis	Human (topical) + veterinary
Neomycin + doxycycline	Aminoglycoside + tetracycline	Human + veterinary
Colistin	Polymyxin E	Human + veterinary
Amoxicillin	Penicillin	Human + veterinary
Levofloxacin	Fluoroquinolone	Human + veterinary
Colistin + doxycycline	Polymyxin E + tetracycline	Human + veterinary
Neomycin + doxycycline	Aminoglycoside + tetracycline	Human + veterinary
Neomycin + oxytetracycline	Aminoglycoside + tetracycline	Human + veterinary
Neomycin + sulphadizamine	Aminoglycoside + sulphonamide	
Avilamycin	Orthosomycin	
Oxytetracycline	Tetracycline	Human + veterinary
halquinol	chloroxine	
Tilmicosin	macrolide	veterinary
Perfloxacin	quinolone	Discontinued in human Used in Veterinary
Tiamulin	Pleuromutilin (inhibit protein synthesis by binding to 50S subunit of ribosomes)	Veterinary
Oxyclozanide	salicylanilide anthelmintic	Veterinary
Enrofloxacin	Fluroquinolone	Veterinary
Chlorotetracycline	Tetracycline	
Ceftiofur	Third generation cephalosporin	Veterinary
Amikacin	Aminoglycoside	Veterinary + human
Tylosin	Macrolide	Veterinary
Flumequine	Fluoroquinolone	Discontinued in human Used in Veterinary

One Health Approach



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WHO-AGISAR Project

Monitoring the Antimicrobial resistance profile of bacterial food borne pathogens in Humans, food animals and retail meat in India

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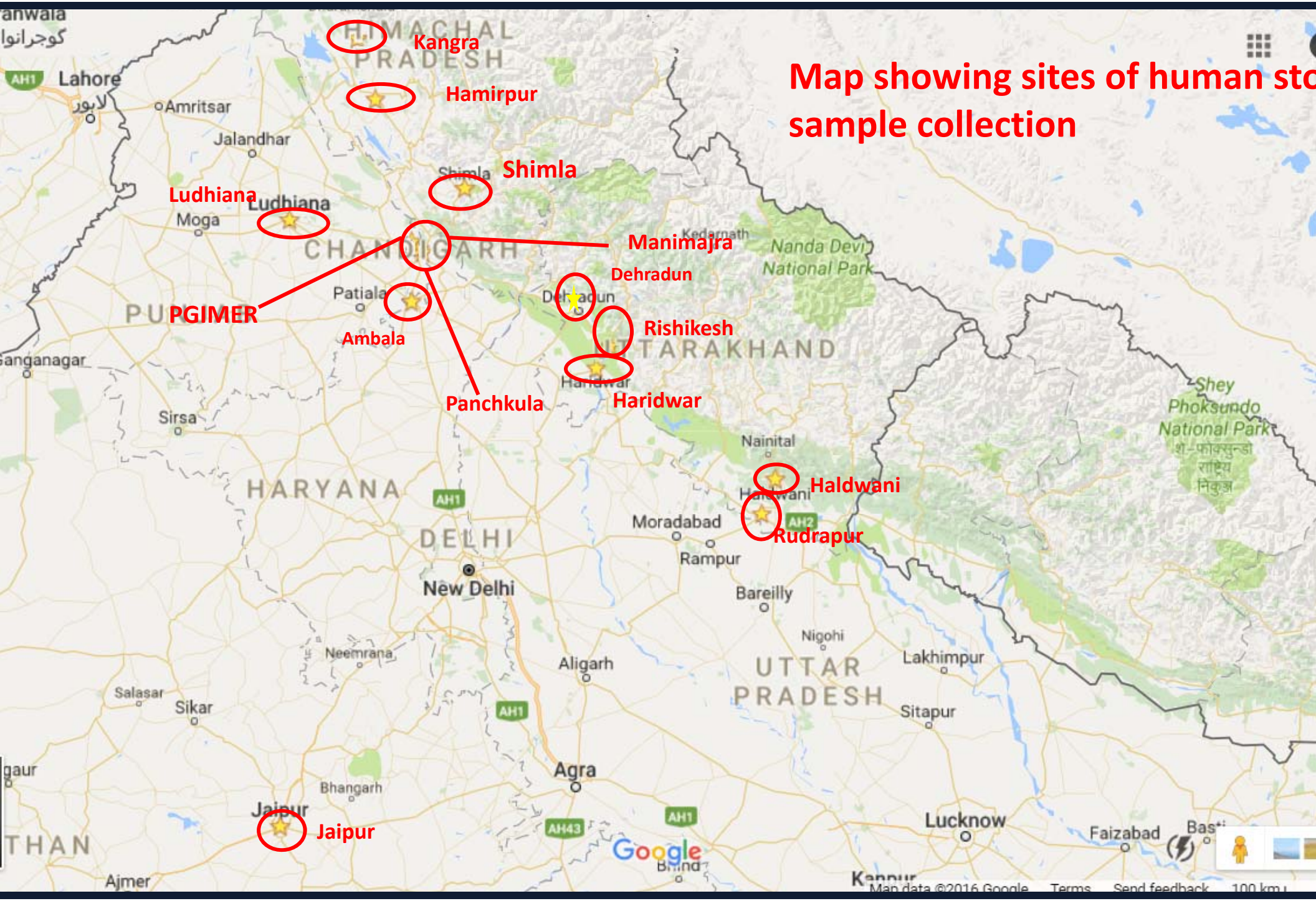
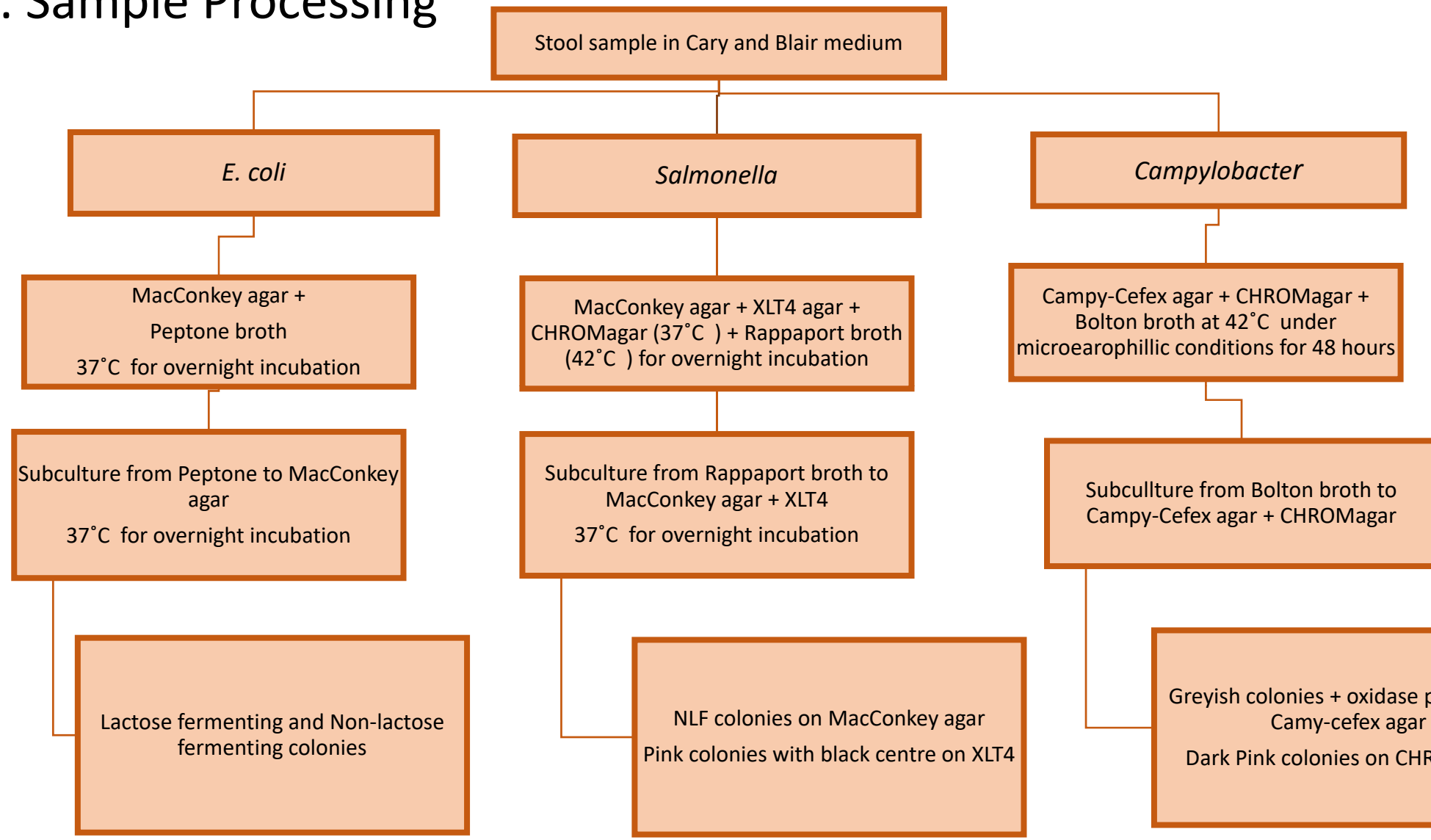
Human Samples

Sample collection

- Total samples collected = 1968
- proforma for history was filled
- Diarrheal stool samples were collected & transported in Cary and Blair transport medium


Map showing sites of human sto
sample collection
Kangra
Hamirpur
Shimla
Ludhiana
Manimajra
Dehradun
PGIMER
Ambala
Rishikesh
Panchkula
Haridwar
Haldwani
Rudrapur
Jaipur
HIMACHAL PRADESH
CHANDIGARH
HARYANA
DELHI
UTTARAKHAND
UTTAR PRADESH
Lahore
Amritsar
Jalandhar
Moga
Patiala
Sirsa
Nainital
Moradabad
Rampur
Bareilly
New Delhi
Aligarh
Agra
Lucknow
Faizabad
Lakhimpur
Sitapur
Nigohi
Neemrana
Salasar
Sikar
Bhangarh
Ajmer
Nanda Devi National Park
Shey Phoksundo National Park
AH1
AH2
AH43
Google
Map data ©2016 Google
Terms
Send feedback
100 km

• 2. Sample Processing

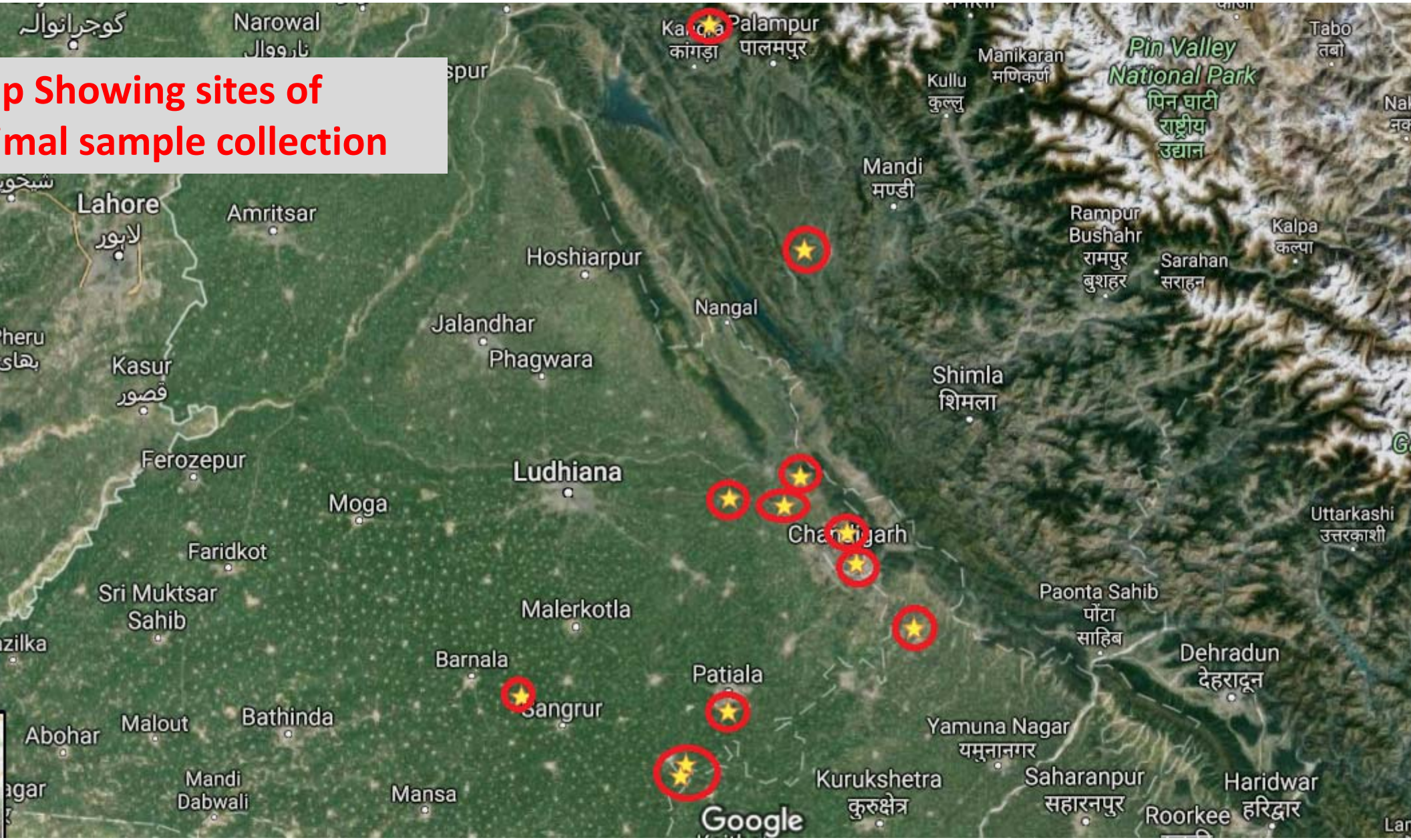


Colonies were identified by MALDI-TOF *

- *Salmonella* identified up to genus level
- Further serotyping to be done

Animal samples

nples collected: 839
ol: 487
at: 352


p Showing sites of
mal sample collection
Narowal
Palampur
पालमपुर
कांगड़ा
Manikaran
मणिकर्ण
Kullu
कुल्लु
Pin Valley
National Park
पिन घाटी
राष्ट्रीय
उद्यान
Tabo
तबो
Mandi
मण्डी
Lahore
لاہور
Amritsar
Hoshiarpur
Rampur
Bushahr
रामपुर
बुशहर
Sarahan
सराहन
Kalpa
कल्पा
Nangal
Jalandhar
Phagwara
Kasur
قصور
Shimla
शिमला
Ferozepur
Ludhiana
Moga
Uttarkashi
उत्तरकाशी
Faridkot
Sri Muktsar
Sahib
Malerkotla
Paonta Sahib
पोंटा
साहिब
Barnala
Patiala
Dehradun
देहरादून
Abohar
Malout
Bathinda
Sangrur
Yamuna Nagar
यमुनानगर
Mandi
Dabwali
Mansa
Kurukshetra
कुरुक्षेत्र
Saharanpur
सहारनपुर
Haridwar
हरिद्वार
Roorkee
Google

Animal samples

Animal (goat, sheep, pig) meat and stool samples were collected from slaughter house, Chandigarh which receives animals from Chandigarh and nearby areas of Panchkula, Manimajra, Mohali and Punjab

Poultry stool samples were collected from farms in and around Chandigarh

These samples were processed for *Campylobacter,* Non-Typhoidal *Salmonella* and *E. coli* detection by culture methods

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nimal sample processing

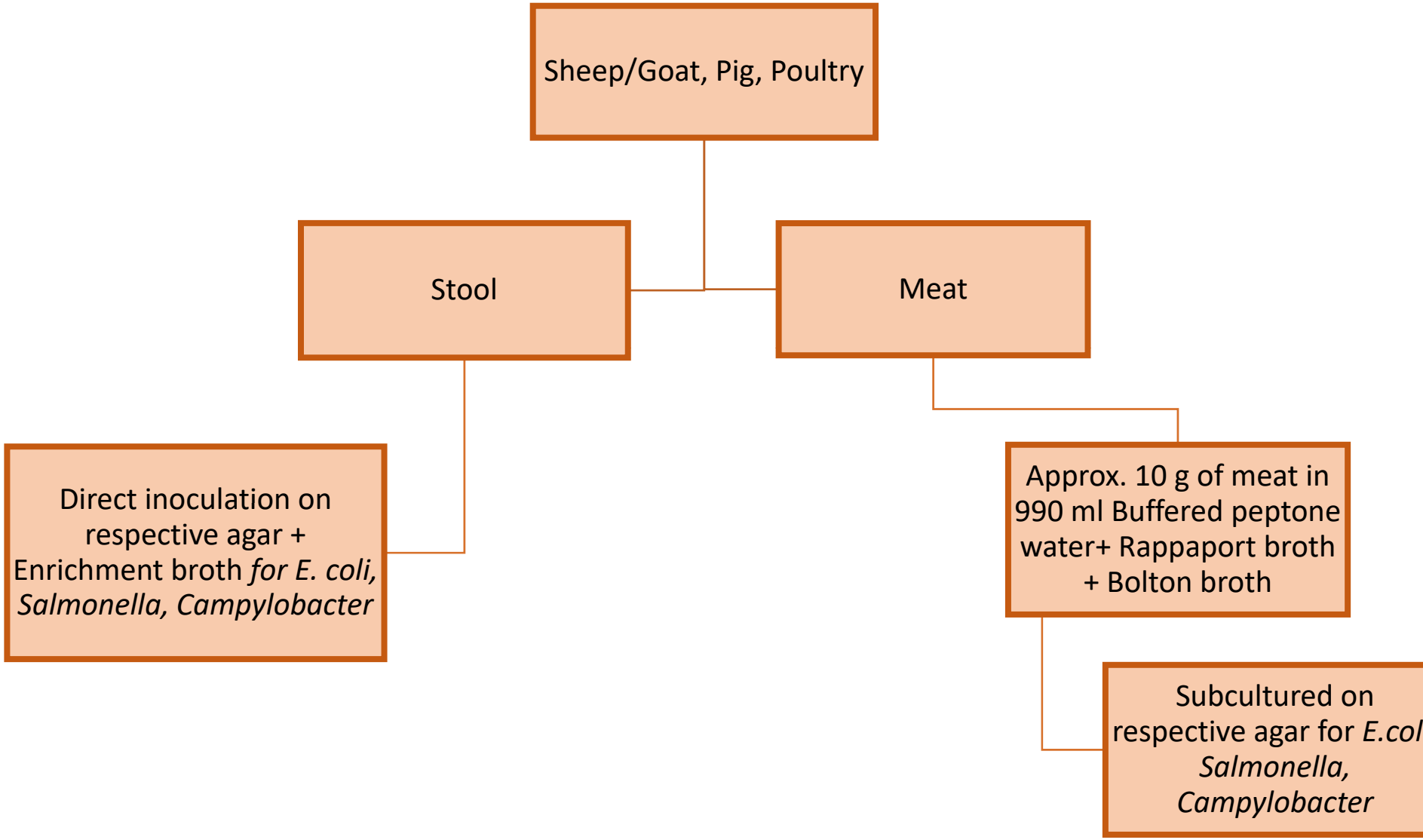


Table showing compiled results from all animals

ource	Sample (n)	Campylobacter (%)	Salmonella (%)	E. coli (%)
oat	Stool (90)	**MALDI identified species** • *Campylobacter jejuni* (38.79%) • *Campylobacter coli* (58.41%) • *Campylobacter hyointestinalis* (2.33%)	7 (7.78)	**Multiplex PCR identified pathotypes** EAEC (1%) EPEC (8%) STEC/EHEC (4.5%) ETEC (5.5%)
	Meat (191)		3 (1.57)	
g	Stool (57)		22 (38.6)	
	Meat (151)		29 (19.21)	
hicken	Stool (340)		38 (11.18)	
	Meat (10)		0	
otal	839		99 (11.8)	

017

5% samples has both *Campylobacter* and *Salmonella*

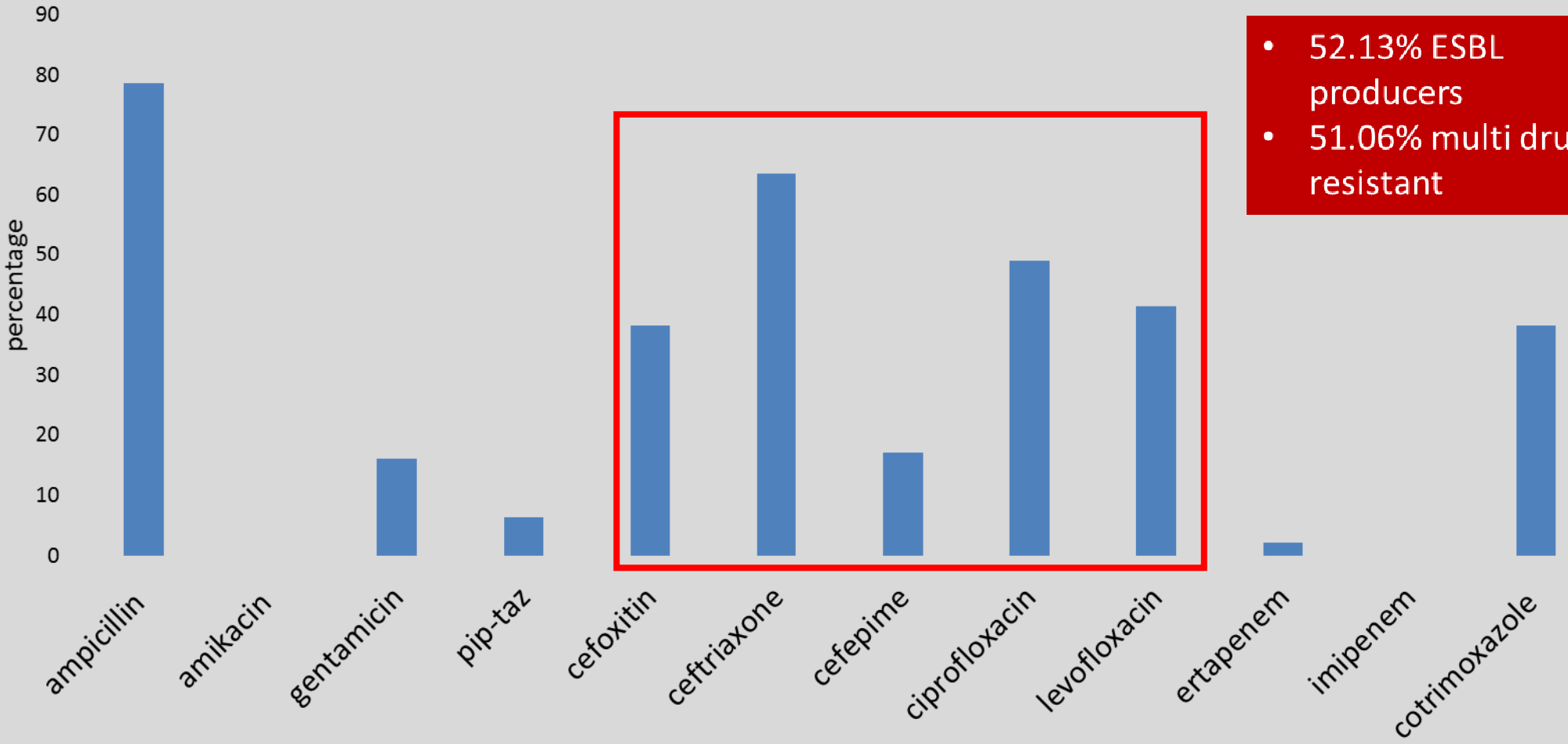
Table showing results of human stool samples

ate	Locations	Number of samples	Campylobacter	Salmonella	DEC E. coli Pathotypes		
					EAEC	EPEC	ETEC
andigarh	PGIMER	850	10	13	36	26	32
ryana	Panchkula	35	4	0	5	6	2
	Manimajra	745	12	9	13	21	23
	Ambala	29	2	0	5	4	4
machal Pradesh	Kangra	8	0	0	0	1	1
	Shimla	70	3	1	7	10	5
	Hamirpur	11	1	0	2	2	3
tarakhand	Rudrapur	19	3	0	2	2	0
	Haldwani	13	0	0	3	1	4
	Rishikesh	86	4	1	6	6	7
	Dehradun	4	0	0	1	0	1
	Haridwar	38	3	0	7	3	6
jasthan	Jaipur	24	3	0	8	1	5
njab	Ludhiana	8	0	0	3	0	1
tal		1941	45 (2.32%)	24 (1.24%)	98 (5.05%)	83 (4.28%)	94 (4.84%

Antibiotic usage and residues

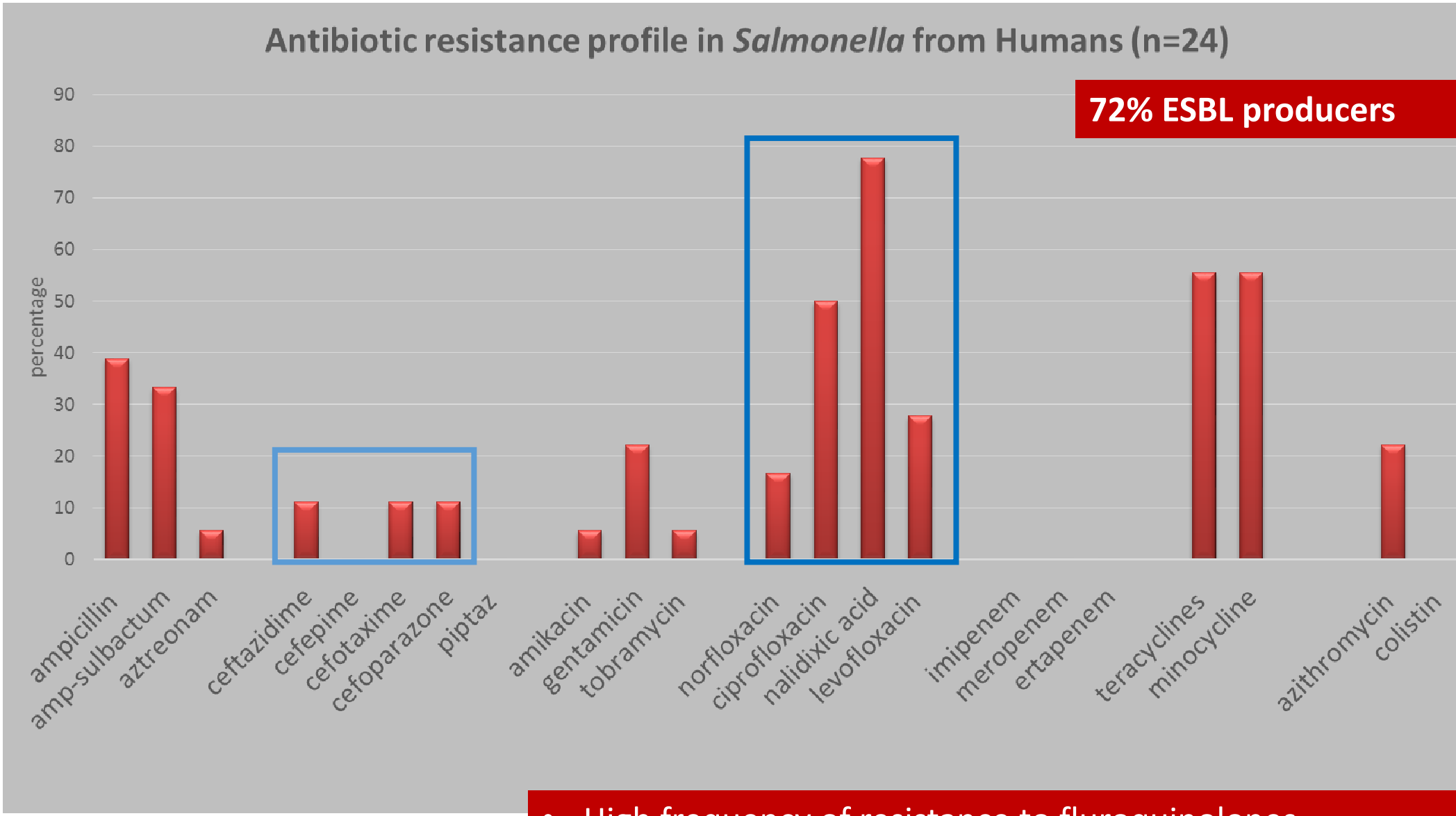
Antibiotic susceptibility

Antibiotic resistance profile of ETEC in humans (n=94)

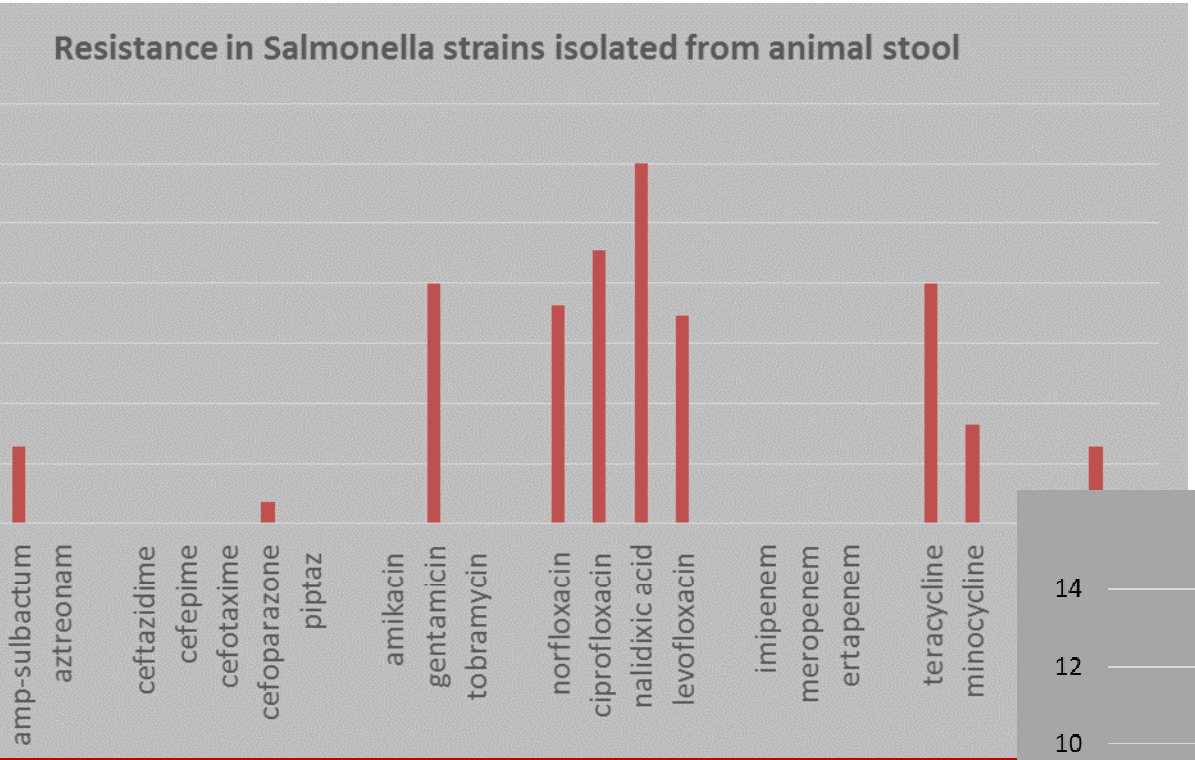


- 52.13% ESBL producers
- 51.06% multi drug resistant

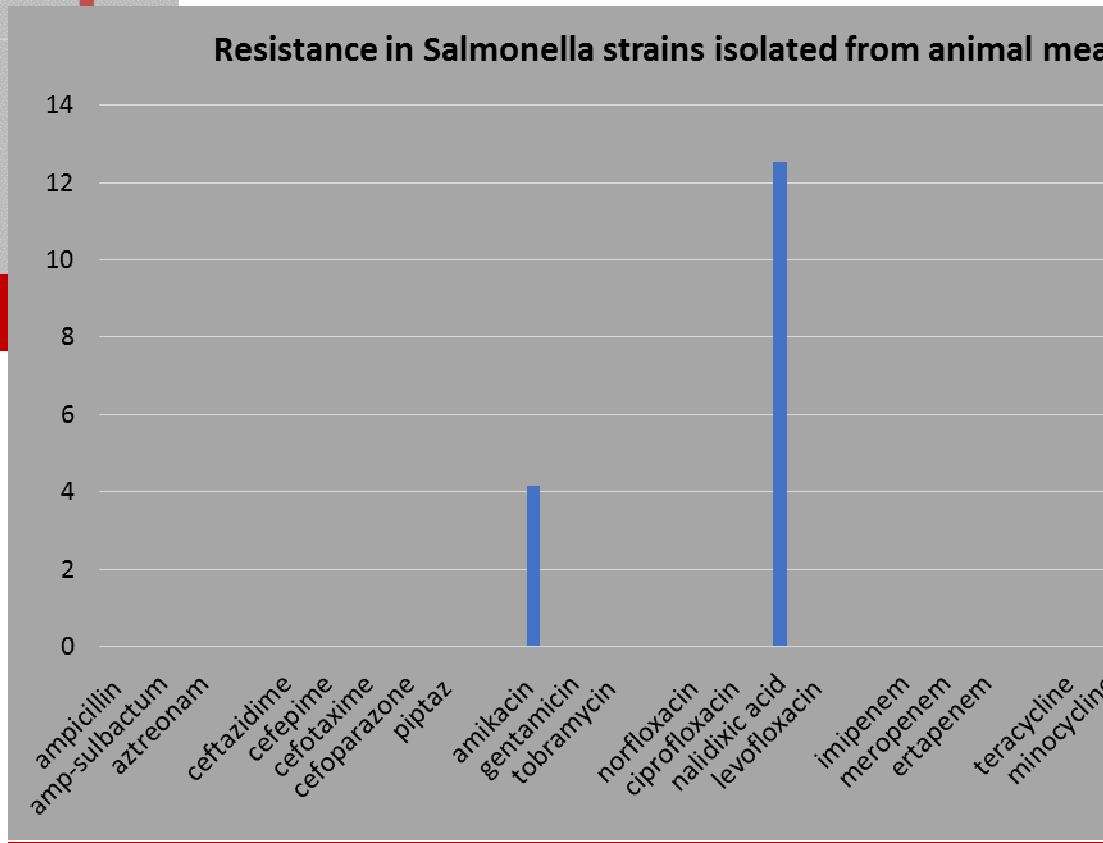
High frequency of resistance to cephalosporins and fluroquinolones



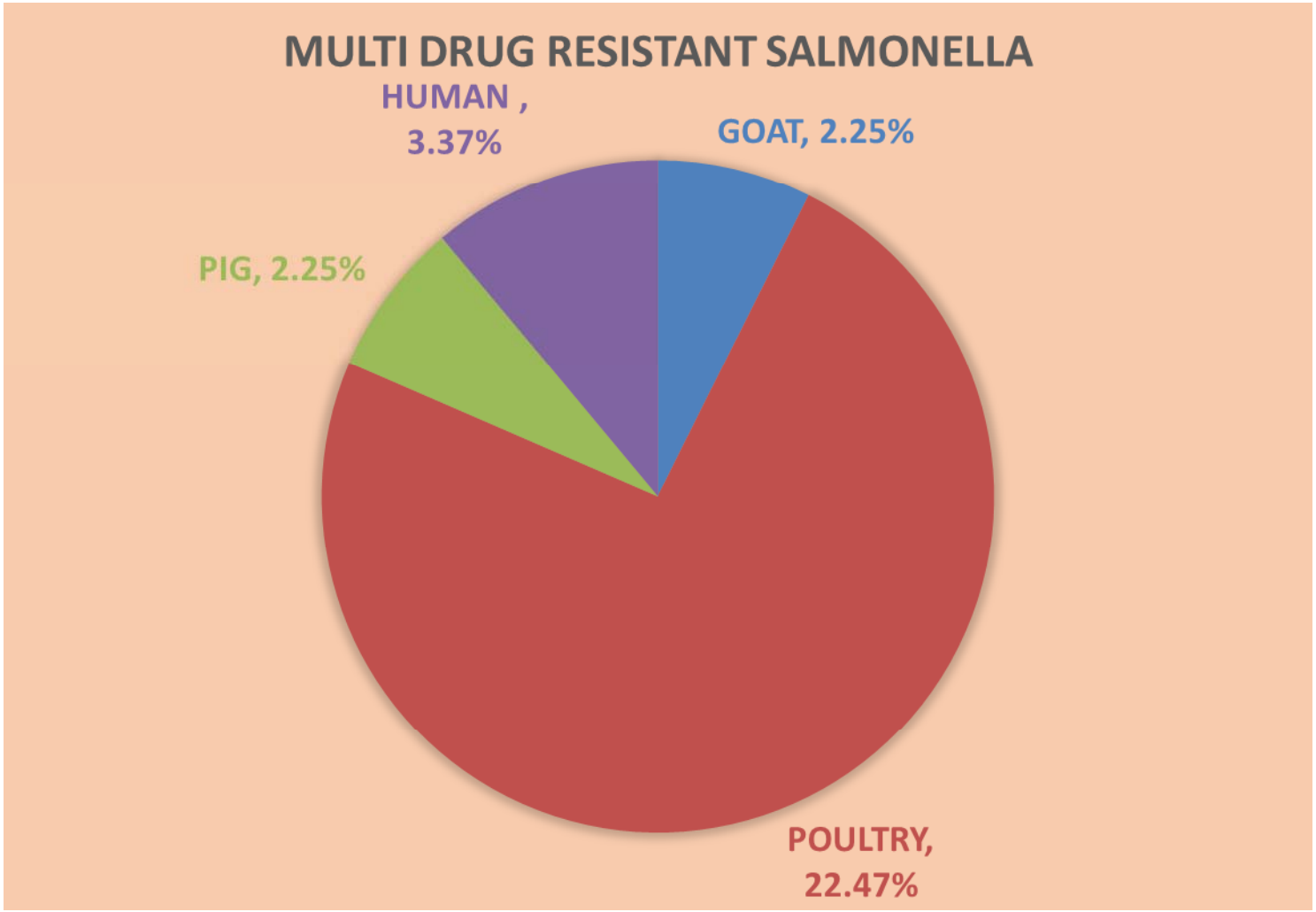
- High frequency of resistance to fluroquinolones
- Resistance seen against 3rd generation cephalosporins
- No resistance to carbapenems

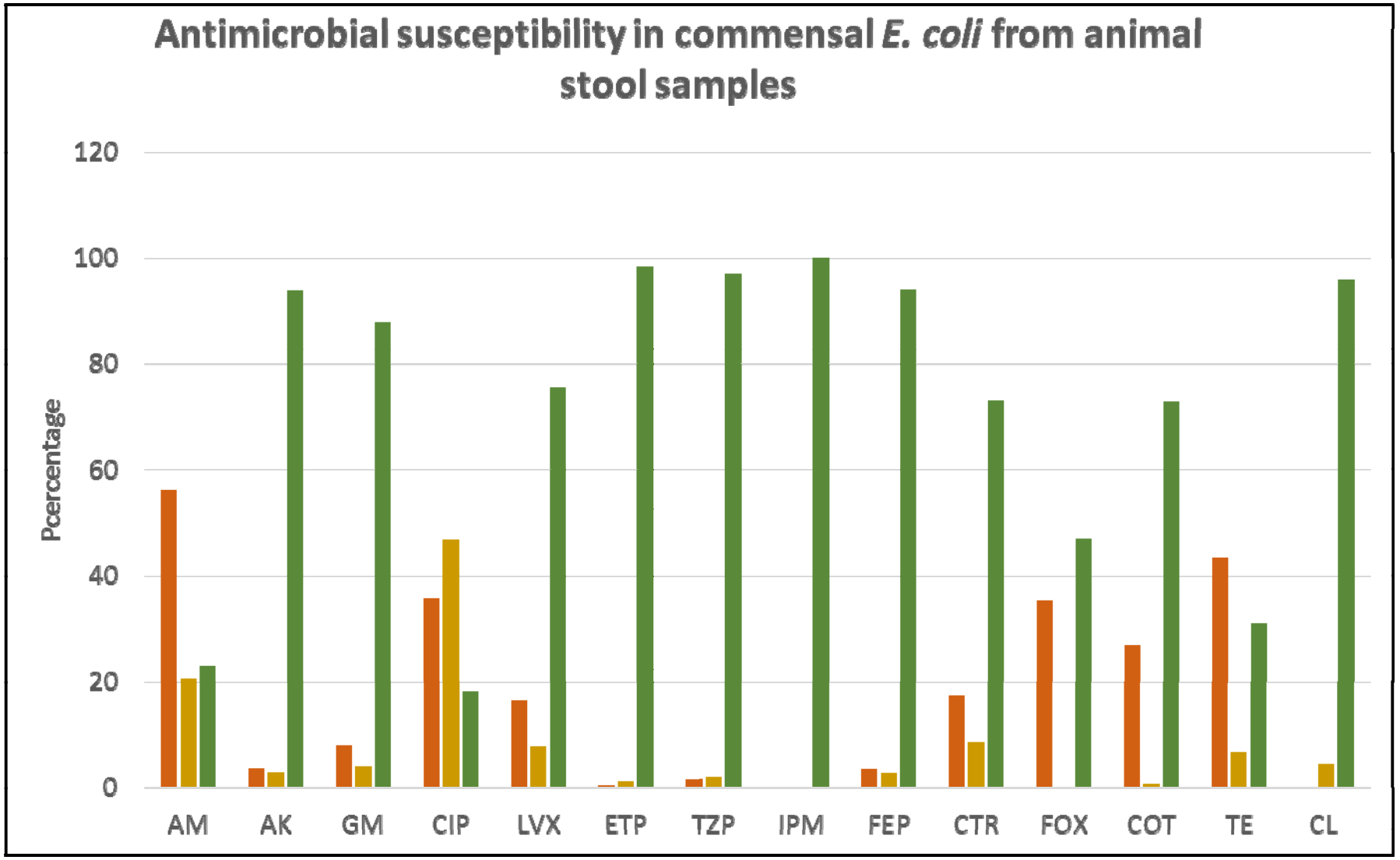


sistance to fluroquinolones



Low resistance was detected


MULTI DRUG RESISTANT SALMONELLA
HUMAN , 3.37%
GOAT, 2.25%
PIG, 2.25%
POULTRY, 22.47%


Antimicrobial susceptibility in commensal E. coli from animal stool samples
Pcercentage
120
100
80
60
40
20
0
AM
AK
GM
CIP
LVX
ETP
TZP
IPM
FEP
CTR
FOX
COT
TE
CL

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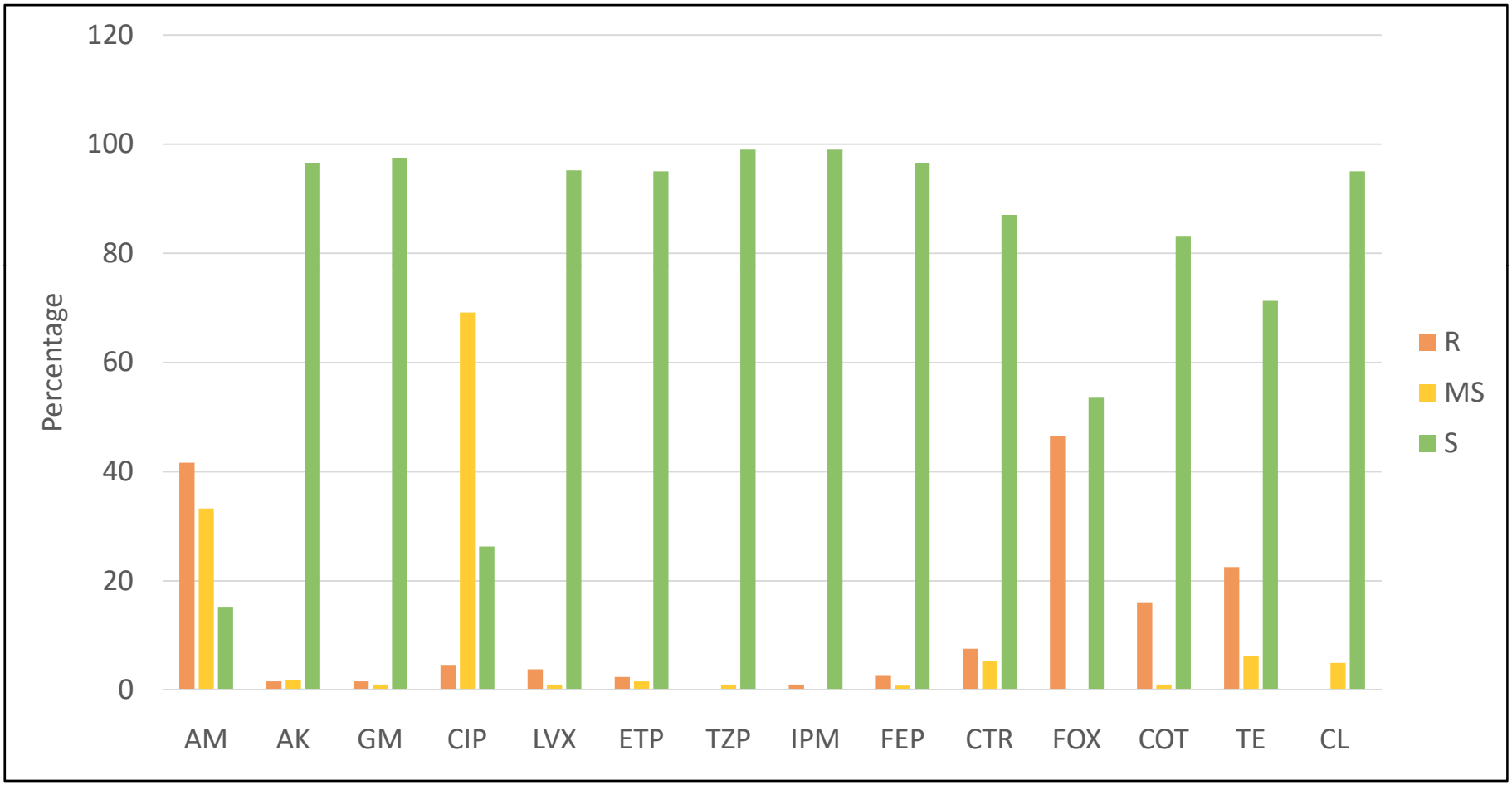
Antimicrobial susceptibility in commensal E. coli from meat samples



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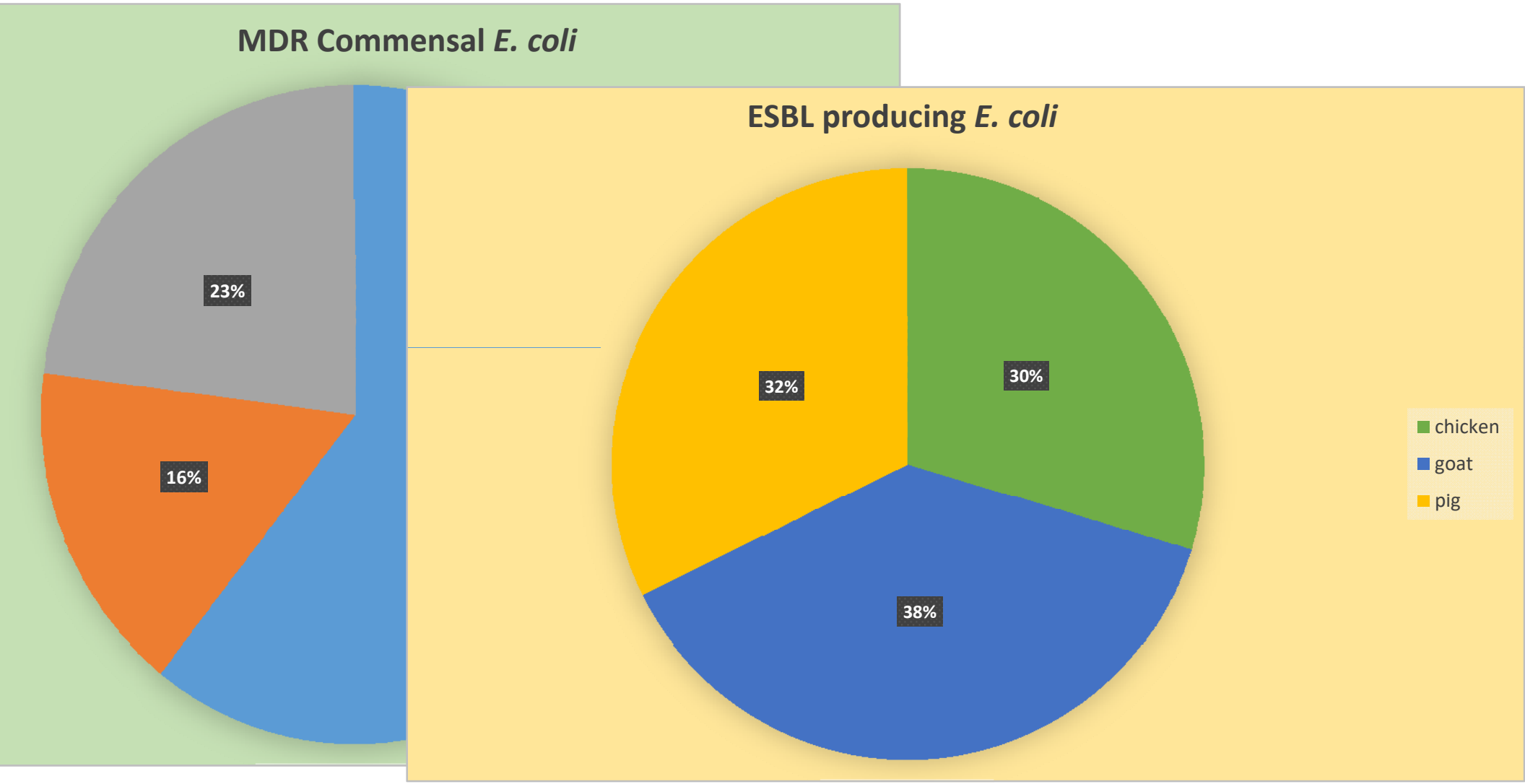
MDR Commensal *E. coli*
23%
16%
ESBL producing *E. coli*
32%
30%
38%
chicken
goat
pig

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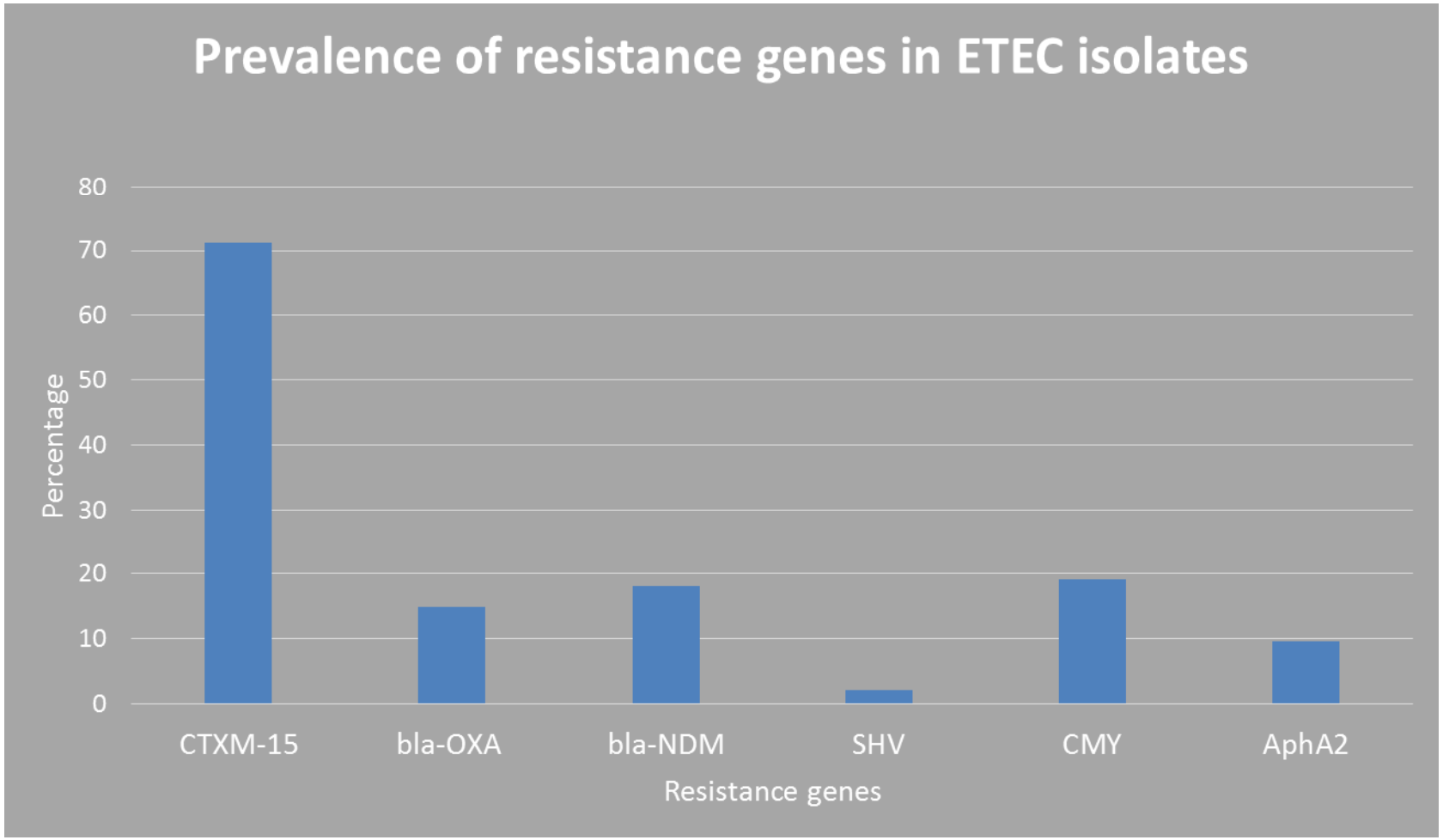
Antibiotic resistance genes


Prevalence of resistance genes in ETEC isolates
80
70
60
50
40
30
20
10
0
Percentage
CTXM-15
bla-OXA
bla-NDM
SHV
CMY
AphA2
Resistance genes

MLST of ETEC isolates

One of the ETEC strain belongs to ST131 complex and some new sequence types have been identified

ST131 has emerged worldwide and is linked to *bla*$_{\text{CTX-M-15.}}$ Extensive studies investigating the association of th multilocus sequence typing (MLST) clonal complex ST131 and *bla*$_{\text{CTX-M-15}}$ have been reported fro Canada, India, Kuwait, France, Switzerland, Portugal, Spain, Korea and Japan; worldwide dissemination of *bla*$_{\text{CTX-M-}}$ seems to be linked to this clonal complex, which is situated in the phylogenetic group B2. This demonstrates th need for constant surveillance in developing countries to prevent the spread of these multiresistant isolates

Another ETEC strain belong to ST 117strains isolated worldwide (such as Brazil, USA, Egypt, Denmark, S Lanka, and South Korea) have ColV related plasmids, were involved in osteomyelitis and arthritis cases

These STs are commonly shared by APEC and human ExPEC strains

These hypothetical hybrid strains could have the potential to infect humans and birds.

Many new STs are found

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Future work

Serotyping of *Salmonella* isolates

Perform MIC testing of *Campylobacter, Salmonella and E. co*
pathotypes isolates using sensititre plates

Estimate Antibiotic residue levels in meat samples by LC-MS-MS

Farm enviorns, water sewage using one health approach

Perform Whole Genome Sequencing of isolates to understand th
transmission dynamics and determine AMR with more precision

Team Members

Dr. Balvinder Mohan, Dr. Chandra Deo, Harpreet, Naveen, Vinay Modgil, **Jaspreet Mahindroo**, Vishal, Varun Shahi, Meenakshi, Yousuf, Pinki Shankar, Ritu Verma, Bhaskar Samaui, Dhananjay

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