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Genetic characterization of *Yersinia enterocolitica* collected from tonsils of slaughtered pigs

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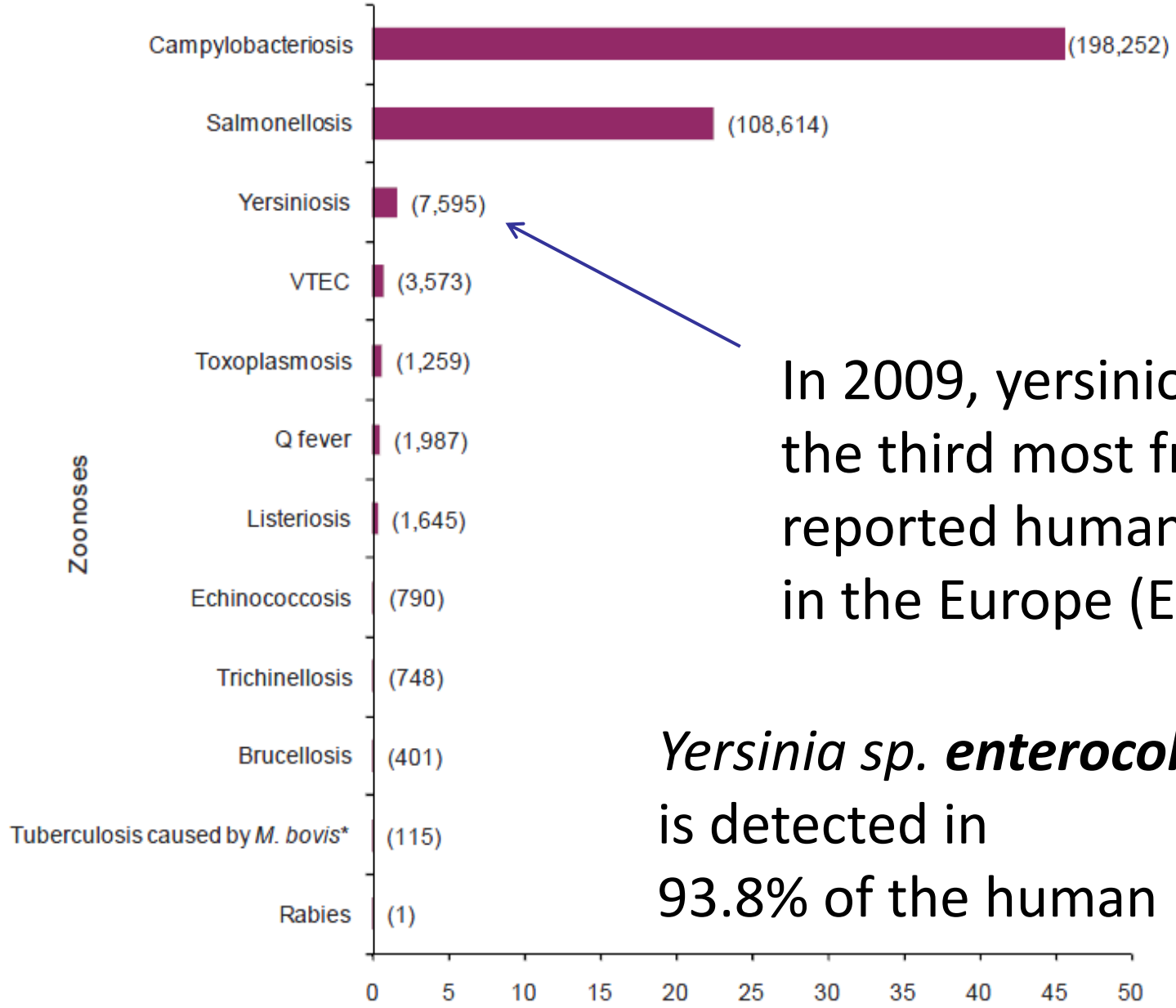
INTRODUCTION



In 2009, yersiniosis was the third most frequently reported human zoonosis in the Europe (EFSA, 2011)

Yersinia sp. ***enterocolitica*** is detected in 93.8% of the human infections.

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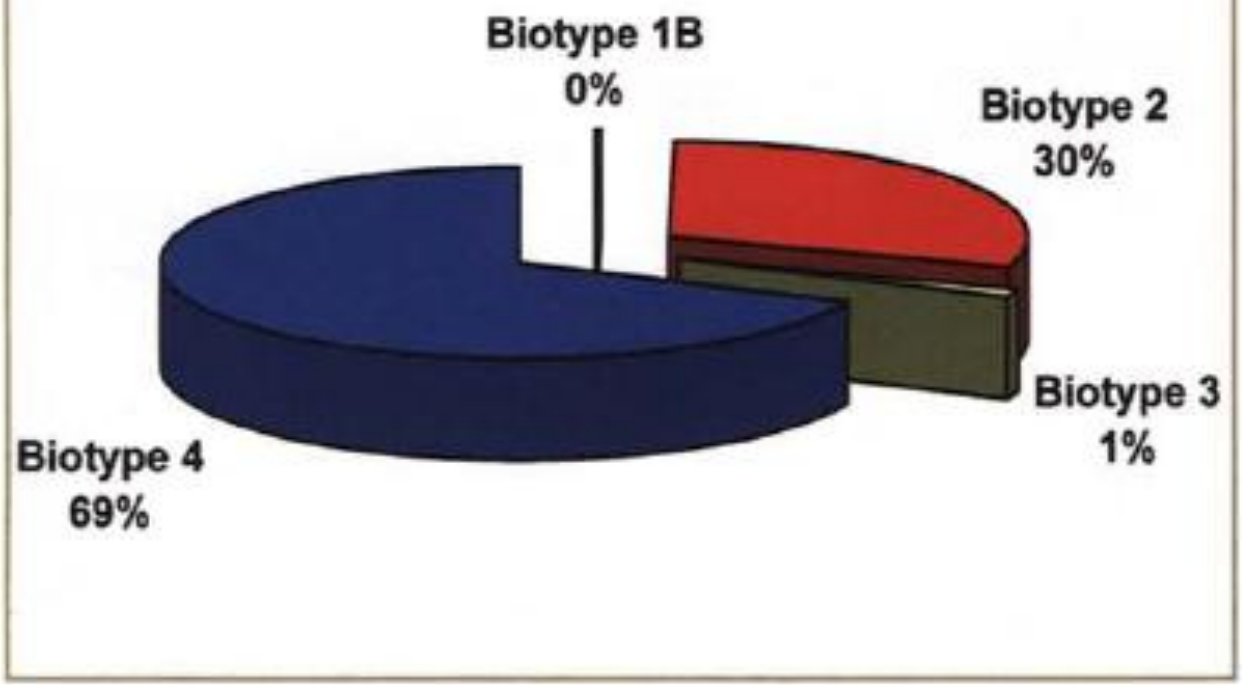
Yersinia enterocolitica : 6 biotypes

1A = nonpathogenic

1B, 2, 3, 4, 5 = pathogenic

Distribution of biotypes
in human infections in France



(Savin and Carniel, 2008)

Pig : principal reservoir of pathogenic *Y. enterocolitica*

Pigs do not develop clinical signs

carry *Y. enterocolitica* in their oral cavity, on tongues and tonsils, and in lymph nodes,

and excrete this bacterium in their feces.

Bioserotype 4/0:3
the most prevalent pathogenic bioserotype isolated from pigs.

Skjerve et al., 1998; Fredriksson-Ahomaa *et al*., 2000; Bonardi *et al*., 2003; Gurtler *et al.,* 2005…….
Kechagiai *et al* ; 2007 ; Laukkanen *et al*; 2009; Van Damme *et al.,* 2010……..

Detection of *Yersinia enterocolitica*

ISO 10273 : 2003 method — Enrichment in 2 broths PSB and ITC
Sreaking on 2 agar plates CIN and SSDC

Biochemicals tests — Confirmation of *Yersinia enterocolitica*
Identification of biotype

Tableau D.1 — Biotypes de *Yersinia enterocolitica*

Biotypes	Tween™-estérase	Esculine	Pyrazinamidase	Indole	Xylose	Tréhalose
1A[a]	+	+	+	+	+	+
1B	+	–	–	+	+	+
2	–	–	–	(+) [b]	+	+
3	–	–	–	–	+	+
4	–	–	–	–	–	+
5	–	–	–	–	D [b]	–

differentiation of pathogenic biotypes from non-pathogenic biotype
some strains not typeable according to the scheme of Wauters *et al.*, (1987).

Virulence genes

Chromosome-encoded virulence factors		
inv	Inv (Invasin) outer membrane protein In all Yersinia spp.,	Invasion into epithelial cells non functional in non-pathogenic nad Y pseudo
ail	Small outer membrane protein Ail YE pathogenic strains	Adhesion and invasion into epithelial cells
ystA	Heat-stable enterotoxin YstA YE, *berco, molla*	Invasion into tissues by damaging the intestinal epithelium
ystB	Heat-stable enterotoxin YstB YE BT1A pathogenic	
myf	major subunit of antigen Myf Mucoid *Yersinia* factor YE pathogenic strains	promote the colonization of the intestine
ure	urease	Increase PH / Acid tolerance of YE
High pathogenic island	Yersiniabactine	Fer captation

Plasmid-encoded virulence factors (70-kb pYV plasmid)		
yadA	Major outer membrane protein Yersinia adhesin A Y pestis, Y pseudo, Ye	Several roles in the virulence
yop	code for at least 14 Yops secreted proteins : YopB, YopD, LcrV, YopE, YopH, YopM, YopO,Yop P, YopT, YopN	Protection against macrophages / apoptosis
vir F	an important transcriptional regulator of other plasmid genes	

Genetic characterization of *Yersinia enterocolitica*

Genetic profiles by typing method

PFGE *Xba*1 Estrada 2011; Baumgartner 2007; Badhuri 2009

NotI Shivonen 2011; Frediksson-Ahomaa 2007; Thisted-Lambert, Danielsson-Tham 2005; Korte 2004

SfaAI, SfiI Zacharczuk 2009

NotI, ApaI, XhoI, SpeI Frediksson-Ahomaa 2011, 2010

AFLP Boghenbor 2006; Fearnley 2005

MLVA Shivonen 2011

PCR ribotyping Estrada 2011

RAPD Blixt 2003

Presence / absence of virulence genes by PCR

virF, ail, inv Martinez 2011

inv, ail, ystA, ystB, ystC, yadA, virF Zheng 2008

virF, ail, ystA, myfA Estrada 2011;

virF, yadA, yst, Kechagia 2007

ail, ystA, myfA, yadA, ystB Kot 2010, 2007

virF, ail, yst, hreP Frediksson-Ahomaa 2011

virF, ail, yst, rfbC Thisted-Lambert, Danielsson-Tham 2005

yadA Korte 2004

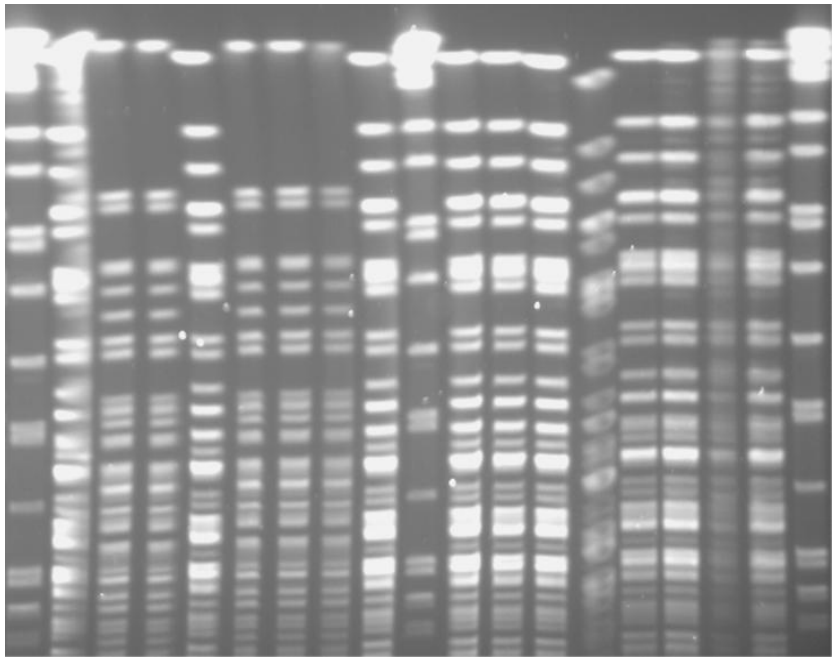
MATERIAL and METHODS

From January to March 2009,
detection of *Yersinia enterocolitica*
from 900 tonsil swabs collected from 45 pig batches
in one slaughterhouse (Fondrevez *et al*., 2010).

316 *Y. enterocolitica* isolates

Biotyping by biochemical tests
as described in the ISO method

Genotyping by RFLP-PFGE
using *Xba1* enzyme

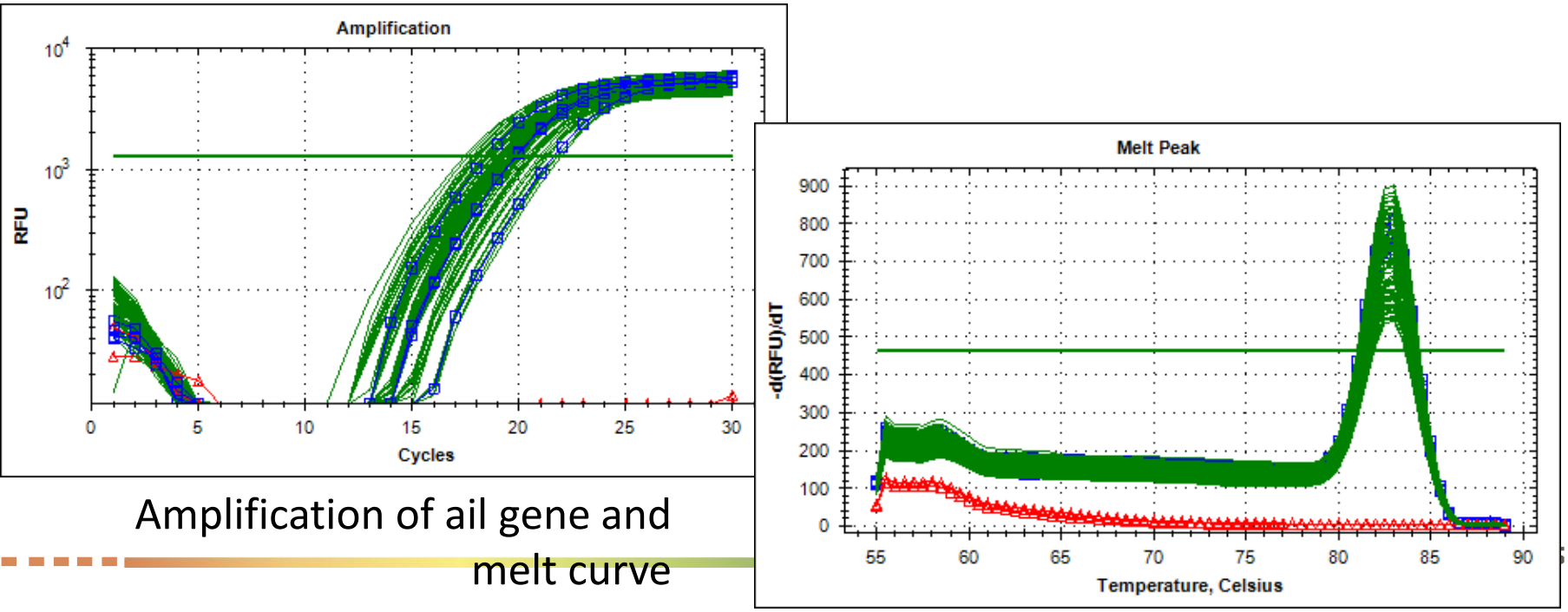


Sybr®Green Real-Time PCRs developed for the detection of plasmid- and chromosome-borne virulence genes to investigate the distribution of 4 genes in our isolates

Using primers published by Ibrahim *et al*., 1997 and Kot *et al*., 2010.

ail, myfA, and ystA carried by the genome

yadA carried by the pYV plasmid.

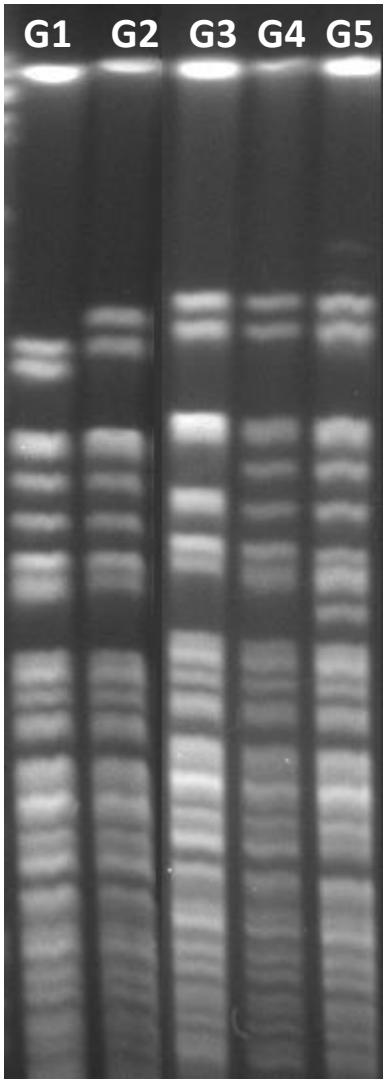


Amplification of ail gene and melt curve

RESULTS

100% of isolates with pathogenic biotypes :

- 85.1% of isolates : biotype 4
- 14.9% of the isolates : biotype 3



5 *Xba*1 PFGE patterns (G1 to G5) very similar

	PFGE patterns					
Biotype	G1	G2	G3	G4	G5	total
Biotype 4	12	47	22	137	51	269
Biotype 3	27	-	-	18	2	45
	12.3%	**14.8%**	**7.0%**	**49.0%**	**16.7%**	316

PFGE patterns not associated with biotype

- G2 and G3 for biotype 4
- G1, G4 and G5 for biotypes 4 and 3

Among the 31 positive pig batches :

- 11 pig batches with one PFGE pattern
- 20 pig batches with 2 to 3 different patterns

3 virulence gene profiles

Biotype	Presence of virulence gene	total
Biotype 4	**ail+, myfA+, ystA+, yadA+**	233
	ail+, myfA+, ystA+, yadA-	35
	ail+, myfA+, ystA-, yadA-	1
Biotype 3	**ail+, myfA+, ystA+, yadA+**	45
	ail+, myfA+, ystA+, yadA-	2
	Total	316

99.7% of isolates with the 3 chromosomal genes, ail, myfA, ystA
one isolate without ystA

88.0% of isolates carried the plasmid gene : 73.7% from biotype 4
14.2% from biotype 3

Among the 31 positive pig batches :

31 pig batches had isolates with the plasmid gene.

12 pig batches had isolates without the plasmid gene.

CONCLUSION

All the isolates confirmed pathogenic by biochemical tests

the most prevalent biotype : biotype 4

similar results with other studies on pigs

5 *Xba*1 PFGE patterns almost similar

➔ A low genetic diversity in this population collected from 31 pig batches

➔ pig batches from the same slaughterhouse

➔ PFGE or *Xba*1 : not enough discriminatory

we are testing now other enzymes *Not*1, *Apa*1

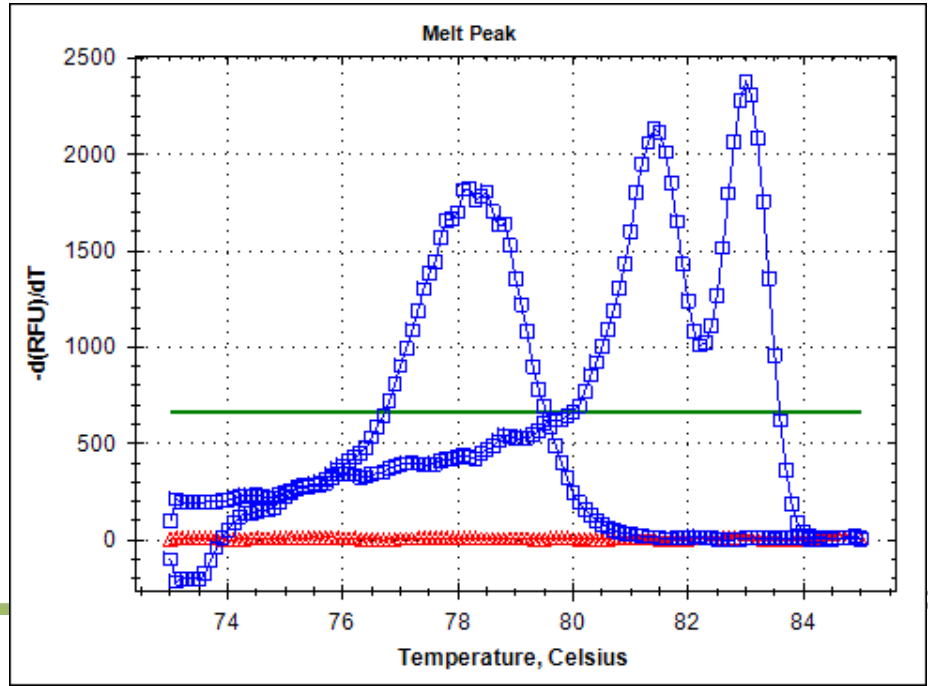
we are looking for another typing method

100% of the isolates carried chromosomal **ail+** and **myfA+** genes
88% of the isolates carried plasmid **yadA** gene

➔ Separate pathogenic isolates from non-pathogenic isolates without doing biochemical tests.

➔ We are testing now PCR on virF gene to confirm the presence of the plasmid

➔ We developed a Real time multiplex-PCR for detecting the 3 genes in one PCR reaction.



Thank you for your attention !