



Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*

Prolonged carriage of extend-
spectrum β -lactamase and AmpC
 β -lactamase-producing
Escherichia coli and *Klebsiella*
pneumoniae in humans:
molecular characteristics and risk
factors

Engeline van Duijkeren, Aniek
Lotterman, Cindy Dierikx, Angela
van Hoek, Paul Hengeveld,
Christiaan Veenman, Alice Florijn,
Lieke Wielders, Jaap van Dissel,
Kitty Maassen, Sabine de Greeff
on behalf of the VGO study group





Transparency Declaration

- The authors have no conflicts of interest to declare




Introduction

- Infections with ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-E/K) are increasing worldwide
- Often preceded by asymptomatic carriage
- Prevalence of carriage in the general population in the Netherlands:
 - 5-10%
 - To date mainly cross-sectional studies
 - Some longitudinal studies on specific groups: travelers & patients
 - Little is known about persistent carriage in humans in the general population



Objective of the longitudinal study

- To determine duration of carriage as well as the characteristics of and risk factors for persistent carriage of extended-spectrum and plasmidic AmpC β -lactamase-producing (ESBL/pAmpC) *E. coli* and *K. pneumoniae* (ESBL-E/K) in predominantly healthy adults in a livestock dense area in the Netherland
- 
- > How long do ESBL-E/K positive individuals remain positive?
 - > If tested positive repeatedly is it with the same ESBL-producing *E. coli* or *K. pneumoniae* (gene, plasmid, bacterium)?
 - > Do negative individuals remain negative?
 - > What is the relevance of single testing?



Material & Methods

Part of the Dutch Farming and Neighbouring Residents' Health (VGO) Study

To be eligible persons had to:

- be between 18 – 70 years
- live in the eastern part of North Brabant or the northern part of Limburg of the Netherlands
- **not** live or work on a farm

Participants were asked to provide:

- One faecal sample: **cross-sectional part**
- 5 faecal samples with one month interval: **longitudinal part**
- fill in questionnaires (6 times)



Material & Methods

ESBL-E/K were isolated and characterized using:

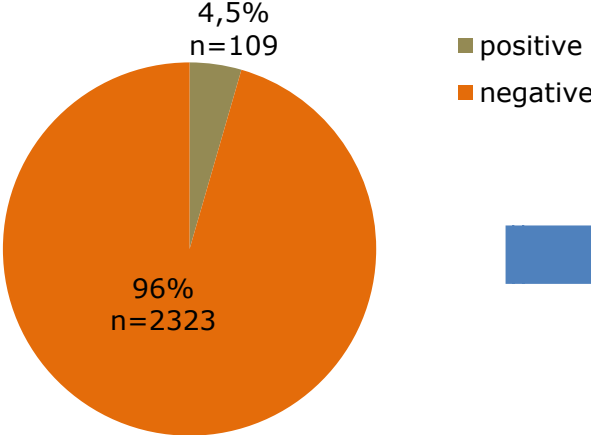
- selective enrichment and culture
- 5 colonies/person/sample moment were tested
- β -Lactamase genes by PCR and sequencing
- *E. coli* and *K. pneumoniae* by MLST
- Plasmid typing by PCR-based replicon typing and pMLST from selection of isolates from persistent carriers

Logistic regression was used to identify risk factors for prolonged carriage and odds ratios (OR) and 95% confidence intervals (95% CI) were calculated.



Material & Methods – Study population

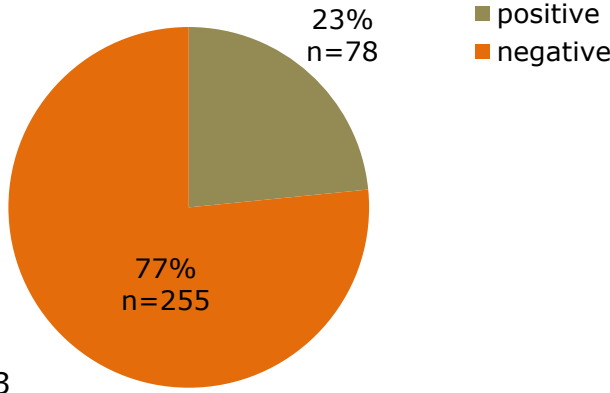
Cross-sectional study



total 2432



Selection longitudinal study



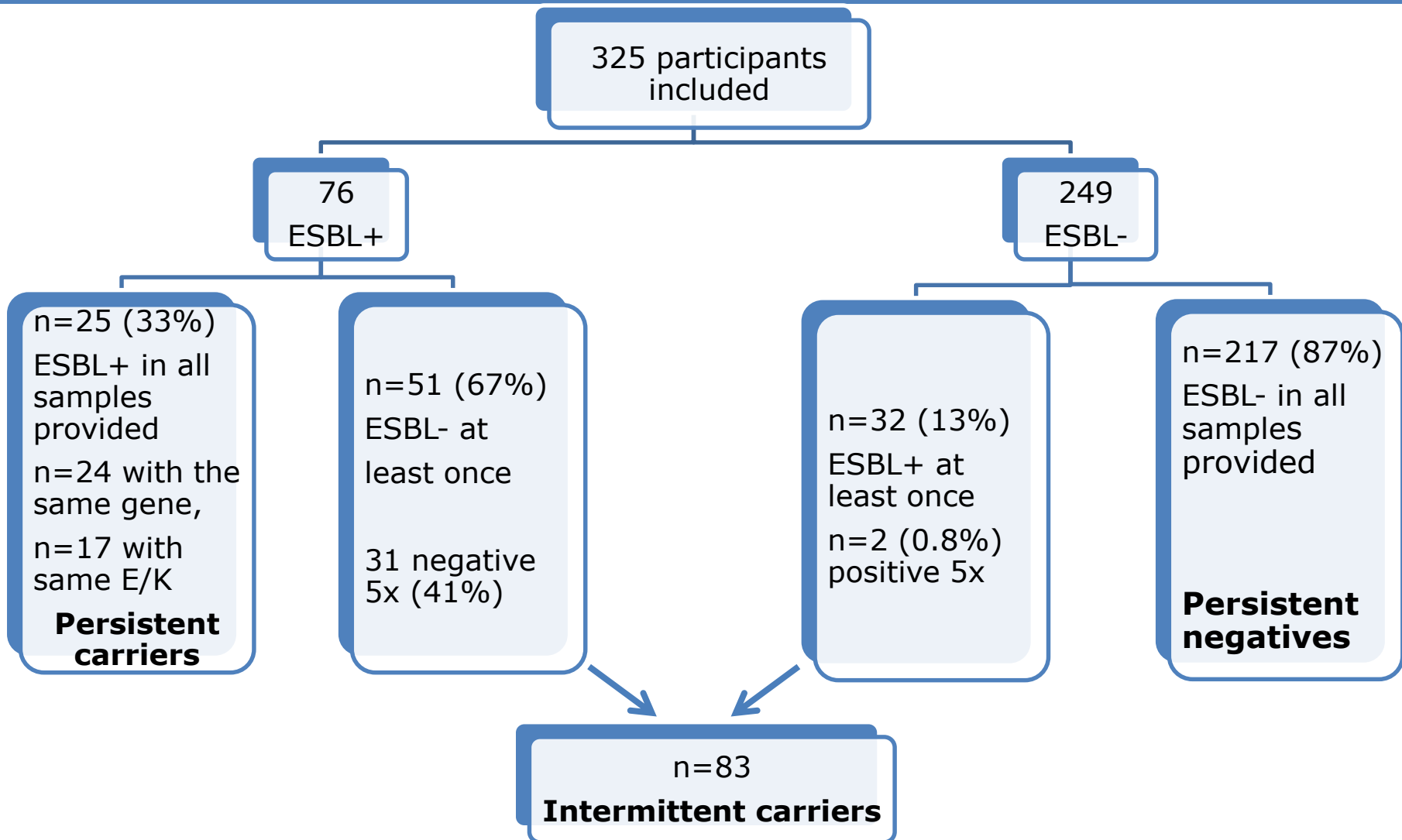
total 333

Wielders et al., 2016. Extended-spectrum β -lactamase- and pAmpC-producing Enterobacteriaceae among the general population in a livestock dense area, *Clinical Microbiology and Infection* 2017 Feb;23(2):120.e1-120.e8.



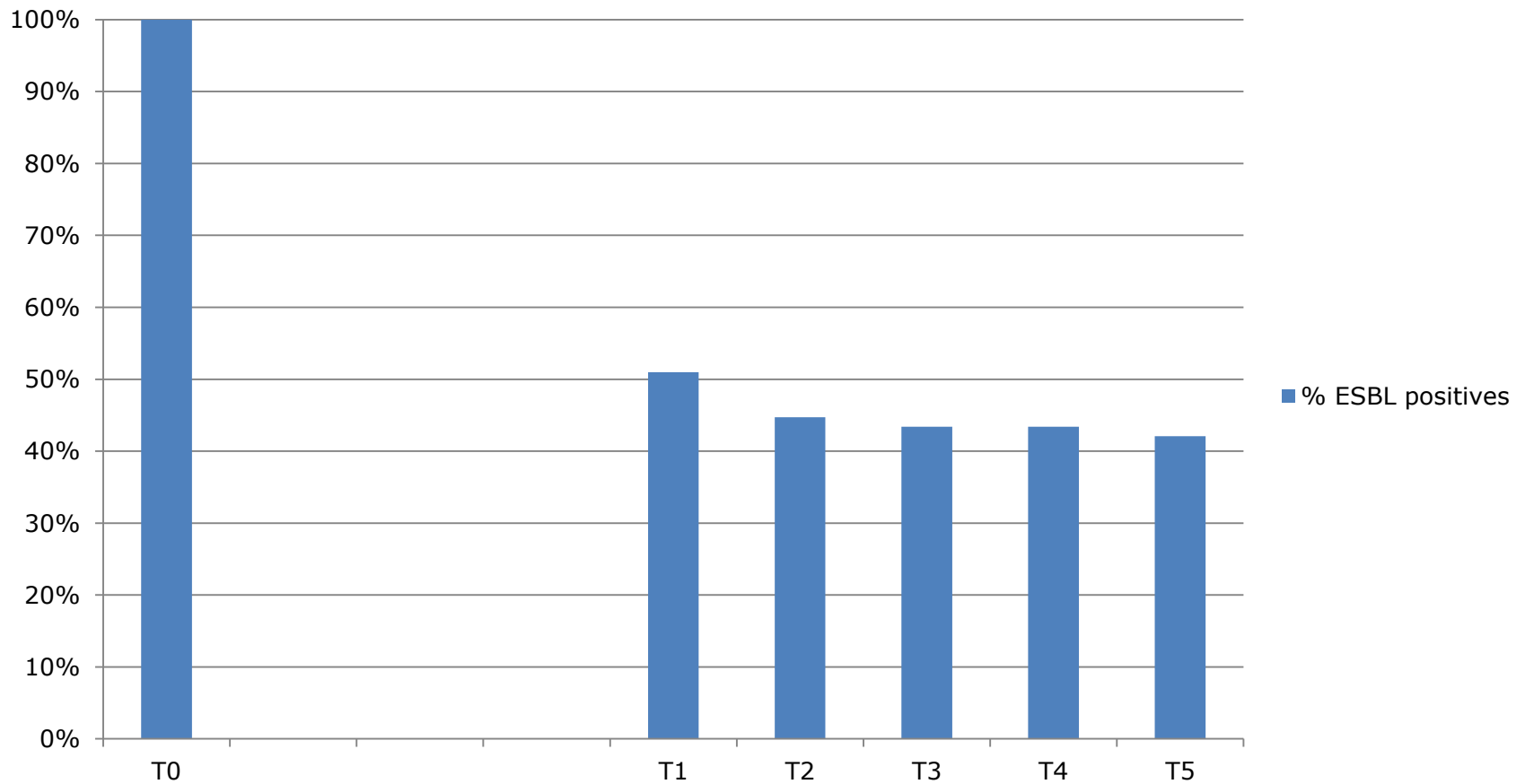
Longitudinal study

- 333 Participants
 - 255 cross-sectional ESBL-E/K negative individuals
 - 78 cross-sectional ESBL-E/K positive individuals
- Cross-sectional study (T0)
- Monthly samples for 5 consecutive months (T1, T2, T3, T4, T5)
- Time between T0 and T1 on average 4 months and between T0 and T5 8 months
- An individual was considered positive for ESBL-E/K if the presence of an ESBL/pAmpC-gene was confirmed
- 8 persons were excluded because less than 4 samples were submitted



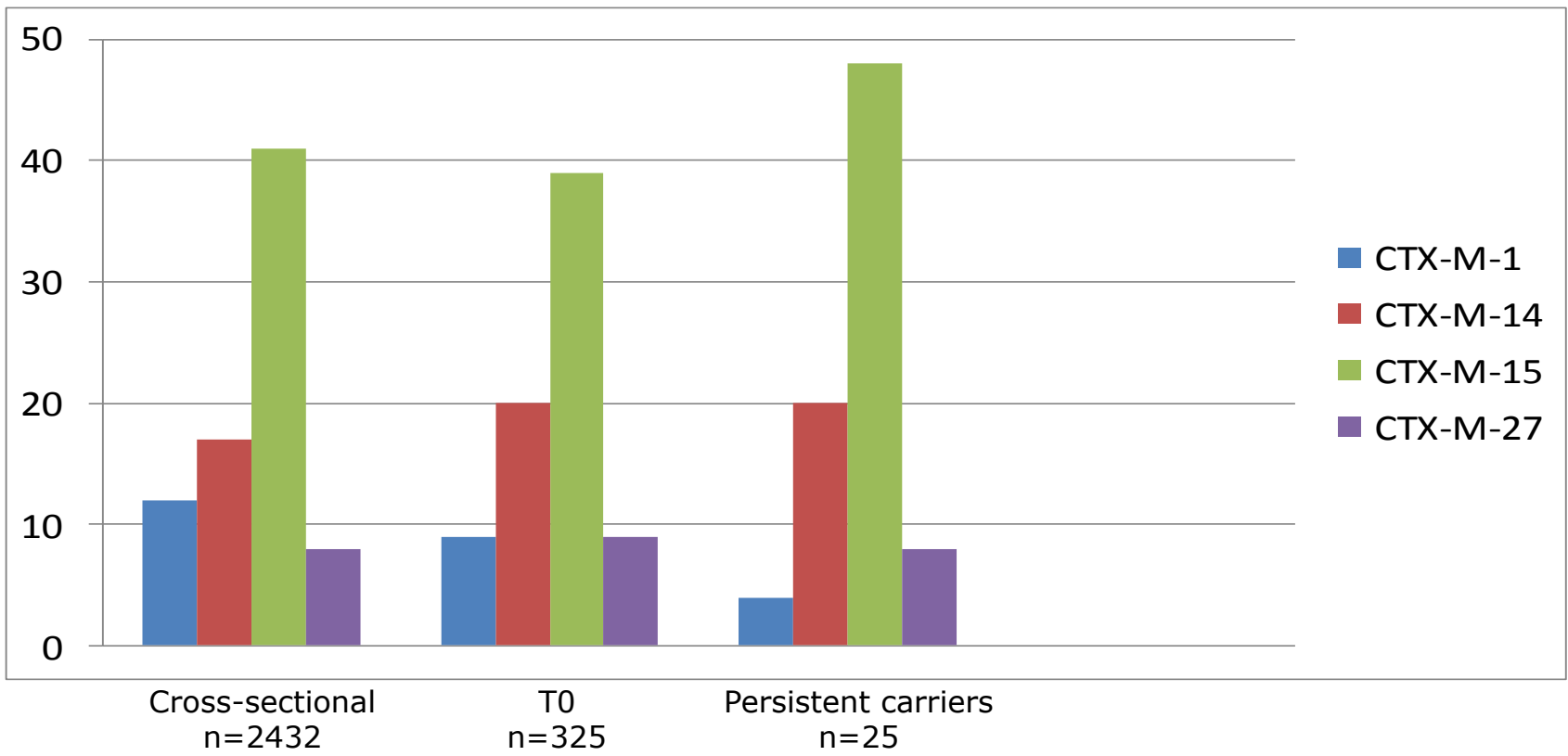


Percentage ESBL-carriage of initially ESBL-positives





Predominant ESBL genes found



Number of participants with identical isolates in all samples provided

<i>E. coli</i> ST 131 / CTX-M-15	3
<i>E. coli</i> ST 131 / CTX-M-27	2
<i>E. coli</i> ST 38 / CTX-M-14	2
<i>E. coli</i> ST 131 / CTX-M-14	1
<i>E. coli</i> ST 648 / CTX-M-14	1
<i>E. coli</i> ST 95 / CTX-M-15	1
<i>E. coli</i> ST 405 / CTX-M-15	1
<i>E. coli</i> ST 501 / CTX-M-15	1
<i>E. coli</i> ST 701 / CTX-M-15	1
<i>E. coli</i> ST 224 / TEM 52	1
<i>E. coli</i> ST 2076 / CTX-M-15	1
<i>E. coli</i> ST 3727 / CMY-2	1
<i>K. pneumoniae</i> ST 902 / CTX-M-15	1
Total	17



Results

- *E. coli* predominated, *K. pneumoniae* was rarely found
- No significant risk factors could be identified for persistent carriage



One example

Sample	T0	T1	T2	T3	T4	T5
Participant 15993		675 / CTX-M-15 incI1 (ST31)		675 / CTX-M-15	675 / CTX-M-15 incI1 (ST31)	675 / CTX-M-15 incI1 (ST31)
	1122 / CTX-M-15 incI1 (ST31)		1122 / CTX-M-15			
				1276 / CTX-M-15		
			1308 / CTX-M-15 incI1 (ST31)			



Conclusions

- 33% (n=25) of the initially ESBL-positives (n=76), remained positive in all subsequent samples (persistent carriers) (8-9 month)
- 41% (n=31) of the initially ESBL-positives (n=76) tested ESBL-negative 5 times, 42% (n=32) were positive at the end of the study
- Nearly all persistent carriers remained positive with the **same gene and plasmid**, but not always in the same strain, indicative of horizontal transmission
- CTX-M-15, CTX-M-14, CTX-M-27 predominated (mainly on incF plasmids)
- *E. coli* ST 131 seems to colonize well, irrespective of the gene
- 87% of the ESBL-negatives remained negative in all consecutive samples
- 0.8% (n=2) initially ESBL-negative participants (n=249) tested ESBL-positive throughout the longitudinal study
- Single ESBL-positive test result provides no accurate prediction for persistent carriage



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