



Report: antimicrobial resistance in commensal *Enterococcus spp.* from poultry, pigs, cows and veal calves, 2013

1 Introduction

Enterococci are regarded as general indicators for resistance amongst Gram positive bacteria, similarly to *E. coli* for the Gram negative bacteria. As with *E. coli* they have the advantage of being present in nearly all animal species, however this is frequently age dependent and the numbers of bacteria are smaller compared to *E. coli*, as reflected in lower isolation successes shown in different surveillance programmes. At the other hand, they are the most prevalent facultative aerobic Gram positive bacteria and as such most suitable for antimicrobial resistance surveillance. Because they are continuously present, they can also be used to follow up resistance evolution in time.

Enterococci are a diverse group of bacteria. They can be divided in species groups. The species mainly involved in surveillances are *E. faecalis* and *E. faecium*. Isolating and recognising these bacteria on enterococcal selective plates, as Slanetz and Bartley agar plates, is not always evident. Species belonging to the *E. faecium* group are difficult to separate and the species frequently co-isolated are *E. hirae* and *E. durans*. The use of a specific PCR only allows detecting a single species or a limited number of species when a multiplex PCR is used. There are no PCRs available allowing the unambiguous identification of the species of the *E. faecium* group. There exists however a well validated PCR technique allowing the identification of multiple species in one test. This technique is t-DNA PCR, which is based on the amplification of the intergenic spacers between the genes encoding t-RNA. Exact sizing of the obtained fragments by capillary electrophoresis and comparison with a database containing the profiles of the different bacterial species allows the unambiguous assignment to a species. However, with the advent of MALDI-TOF equipment allowing the fast and cheap identification of bacteria, identification may become more cost-effective and may be easily executed by first line laboratories that have acquired the equipment.

Enterococci have been studied frequently in other countries. This will allow the comparison of antimicrobial resistances in enterococci from different geographical regions. The genetic background or resistance in this species is also quite well known allowing a scientific interpretation of the resistance data.

2 Materials and Methods

2.1 Sampling

Samples from faecal material were taken from 4 animal categories: broiler chickens, pigs, bovines (for meat production) and veal calves. Samples were taken by samplers of the Belgian Food Agency.



2.1.1 Poultry

Caecal content of broiler chickens was taken at slaughter together with the samples in the framework of *Salmonella* control programme. Caeca from 10 animals were collected and pooled. One sample originated from one farm.

2.1.2 Pig

Pooled fresh faecal material of at least ten animals of approximately 6 months old was collected from slaughter pigs at the abattoir. One sample originated from one farm.

2.1.3 Bovines

Pooled fresh faecal material was collected from the floor of barns harbouring bovines for meat production of less than 7 months of age. One sample originated from one farm.

2.1.4 Veal calves

Pooled fresh faecal material was collected at the abattoir from veal calves of less than 7 months of age.

2.2 Isolation and identification

As compared to 2011, methodology of isolation has been changed to increase the isolation success. At first faecal material was inoculated into a 7% NaCl supplemented BHI broth. One loopfull of this broth was then inoculated on Slanetz and Bartley agar plates and incubated at 37°C for 18-24 hours at DGZ or ARSIA. Next to that, the number of samples taken was increased.

Plates were then transferred to CODA-CERVA where the colonies with an enterococcal morphology were purified on blood agar plates and Slanetz and Bartley plates and incubated at 37°C for 18-24 hours.

Based on their growth aspects on both Slanetz and Bartley and blood agar plates, colonies were selected from identification. DNA was extracted using the alkalic extraction method and was stored at -20°C for further processing. t-DNA intergenic spacer PCR was performed and obtained fragments were sized using capillary electrophoresis on a Beckman CEQ8000 sequencer (Baele et al., 1998). Alternatively MALDI-TOF analysis was performed.

Obtained fragments were compared to the constructed database and strains were identified. *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae* and *Enterococcus durans* were taken into account.

2.3 Susceptibility testing

From a fresh culture on Columbia agar with 5% sheep blood, susceptibility was tested using a micro broth dilution method (Trek Diagnostics). To this end, 1 to 3 colonies were suspended in sterile distilled water to an optical density of 0.5 McFarland. Ten microliter of this suspension is inoculated in 11ml cation adjusted Mueller Hinton broth with TES buffer.

Fifty microliter of the Mueller-Hinton broth with bacteria was brought on a micro-titer plate with the antimicrobials lyophilised, the NVL76 plate as produced by Trek Diagnostics, using the auto-inoculating



system of Trek Diagnostics. The concentrations tested are indicated in table 1 (grey zones are the concentrations tested).

Plates were incubated 18-24 hours at 35°C and read. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected. MICs were semi-automatically recorded by the Trek Vision system using the SWIN software. Results were automatically exported to an Excel file.

Table 1 shows the antimicrobials tested and their abbreviations. Concentrations tested are shown in table 2.

2.4 Analysis of data

Since isolation method was adapted, isolation successes from 2011 and 2012 were compared using Pearsons chi-square test to allow deciding on the inclusion of species.

Data were exported from the Excel file to an Access file in which the number of strains having an MIC for a certain antibiotic were calculated. These data were set in a table that was subsequently exported to an Excel file. In this file breakpoints based on the EUCAST ECOFFs were indicated.

The number of resistant strains was counted and resistance percentages were calculated. Exact confidence intervals for the binomial distribution were calculated using a visual basic application in Excel. A 95% symmetrical two-sided confidence interval was used with $p=0.025$. The lower and upper bound of confidence interval for the population proportion was calculated.

Based on the Pearsons chi-square test, and where appropriate the Fischer exact test, significance of the differences were calculated. As for the differences between years, the chi square test has been used. It should be noted that differences seen here are not an indication of a trend. Trend cannot be calculated yet, since this needs 3 measurement points.

Multi-resistance was determined by transforming the MIC data into resistant (R) and susceptible (S) using ECOFF breakpoints as provided by EUCAST. Number of antimicrobials to which a strain was resistant to was counted and cumulative percentages were calculated. The modal number of antimicrobials to which 50% of the strains was resistant was calculated. Graphical representations were prepared.

3 Results

Results are shown in tables 2 to 39 and figures 1 to 28.

The results are split up into the different animal species and different bacterial species. The division per bacterial species is because normal susceptibility of each may differ. Analysis per animal species allows determining differences between the animal species. Data are discussed only if a sufficient number of strains was obtained.



3.1 Poultry

A total of 184 enterococci from poultry were tested for susceptibility which is only half of the number tested last year. Seventy one were *E. faecalis*, and 113 *E. faecium*. Compared to 2012, there were quite fewer *E. faecalis* (2012: 149) but more *E. faecium* (2012: 63) strains isolated. It is clear that there is a large variation in isolation to be expected from one year to another. It is known that the enterococcal flora of poultry is instable at young age, with high numbers of *E. faecalis* at the age of less than one week, and gradually evolving toward a flora containing more species of the *E. faecium* group (*E. faecium*, *E. hirae*, *E. durans*) and from 6 weeks on (not relevant for broilers but important for layers) it changes to a flora containing mainly *E. cecorum*. Nevertheless, sample size is not sufficient for attaining the set 170 strains of *E. faecium* and *E. faecalis*.

In *E. faecalis* resistance was seen against all antibiotics except gentamicin, vancomycin and linezolid. Resistance was mainly seen against tetracycline, erythromycin and streptomycin with at least half of the strains being resistant and nearly 90% of the strains being resistant to tetracycline. Resistance against the other antibiotics was less than 11%, with for most antibiotics a resistance prevalence of less than 5%.

No resistance was found to vancomycin while resistance against an antibiotic group also formerly used as growth promoter, and still in use as a coccidiostat, are the ionophores (of which is salinomycin is tested here) was as high as 11,1%.

In *E. faecium*, no resistance against florfenicol, linezolid was seen. As for *E. faecalis*, resistance against tetracycline, erythromycin and streptomycin was high, and an additional high prevalence of resistance was found against synergid (quinupristin/dalfopristin), streptomycin, salinomycin and ampicillin. Against the other antibiotics tested resistance was very low. There was one strain vancomycin resistant.

As a general, one can state that for the antibiotics against which resistance is high (erythromycin, tetracycline and streptomycin), it is so for the 2 species tested. In general, prevalence of resistance in *E. faecalis* is significantly lower compared to *E. faecium*. This is significant for the antibiotics ampicillin, salinomycin, and quinupristin/dalfopristin (synergid).

A bit more than 10% of the *E. faecalis* strains were fully susceptible, and for *E. faecium* only 3.5% of the strains were fully susceptible. Approximately half of the *E. faecalis* strains were resistant to two antibiotics while for *E. faecium*, half of the strains were resistant to 4 different antibiotics.

One *E. faecalis* strain was resistant up to 6 antibiotics and one *E. faecium* strain up to 7 different antibiotics. The *E. faecalis* strain remained susceptible to chloramphenicol, ciprofloxacin, florfenicol, linezolid and vancomycin and was resistant to ampicillin and gentamicin, two antibiotics frequently combined in the treatment of severe enterococcal infections. The vancomycin resistant *E. faecium* strain had a low MIC, not indicative for the presence of the classically encountered *vanA* gene. This strain remained susceptible to ampicillin and gentamicin. None of the ampicillin resistant *E. faecium* strains was resistant to gentamicin. The two gentamicin resistant *E. faecium* strains remained susceptible to ampicillin and were resistant to 4



other antibiotics. Eleven strains had an MIC of ciprofloxacin just above the breakpoint. It is not clear whether this represents true resistance or is a breakpoint issue.

Resistance against chloramphenicol, an antibiotic not used anymore is low, with only 3 *E. faecalis* strains being resistant.

This year, no linezolid resistant strains were isolated. Striking remains the high prevalence of resistance against salinomycin, especially in *E. faecium*.

3.2 Pigs

A total of 82 strains from pigs were tested. This is less than one third of what has been tested last year. Only 13 *E. faecalis*, 69 *E. faecium* were included. *E. faecalis* isolation from pigs has always been problematic due to its low prevalence, and for *E. faecium*, it is variable and depending on the presence of the other species of the *E. faecium* group on the plates.

In *E. faecalis* from pigs, most resistance was seen against erythromycin and tetracycline, and then followed by streptomycin and chloramphenicol. These were also the only antibiotics against which resistance has been detected, however, it should be noted that only low numbers of strains were tested.

In *E. faecium*, the number of strains tested is substantially higher compared to *E. faecalis* and the resistance prevalence is estimated more accurately. Here we see resistance against all antibiotics but chloramphenicol and florfenicol. Highest resistance is present against quinupristin/dalfopristin with nearly 90% of the strains being resistant. Next is resistance to tetracyclines (though only 30% of the strains are resistant). Against the other antibiotics, resistance is lower than a quarter of the strains, with for most antibiotics lower than 5%. Resistance against the clinically important antibiotics ampicillin is more than 11%. Gentamicin and vancomycin resistance remains low. The two vancomycin resistant strains have also low MICs, not indicative for the *vanA* gene. It should however be noted that there were significantly more chloramphenicol resistance *E. faecium* compared to *E. faecalis*, however, the number of *E. faecalis* strains tested was very low.

There were only 2 *E. faecium* (2.9%) strains were fully susceptible, though nearly half of the *E. faecalis* (46.2%) were fully susceptible. Seen the low number of *E. faecalis* tested, the high percentage of fully susceptible strain should be interpreted with care, especially seen that last year, only 13% of the strains were fully susceptible. The resistance of the *E. faecium* strains is mainly caused by the fact that most strains were resistant to quinupristin/dalfopristin. Most strains were resistant to only one antibiotic. In *E. faecium* three strain was resistant to 6 antibiotics. Two of them were resistant to ampicillin, of which one resistant to gentamicin, two antibiotics important in the therapy of *E. faecium* infections in humans. These strains remained susceptible to vancomycin. Five of the six strains with resistance to 5 antibiotics were resistant to ampicillin.



3.3 Veal calves

From veal calves 46 *E. faecalis* strains and 107 *E. faecium* strains were tested, which is similar to what has been tested last year.

Resistance in *E. faecalis* was highest for streptomycin, tetracycline and erythromycin followed closely by chloramphenicol. The only antibiotic against whom no resistance was found was linezolid. Other resistances were low.

Resistance in *E. faecium* was mainly seen against the antibiotic quinupristin/dalfopristin. Other resistances were approximately 50% against tetracyclines and erythromycin and streptomycin (39%) and ampicillin (18%). For the remaining antibiotics resistance remained below 5%. Three strains were resistant to vancomycin, two with low MICs and one with a high MIC. Nineteen strains were resistant to ampicillin and this represented a bit more than 17% of the strains. Gentamicin resistance was seen in 3 strains.

Approximately 60% of the *E. faecalis* strains were resistant to three and more antibiotics. Less than 10% of the strains remained fully susceptible. Four strains were resistant to 6 antibiotics. Two of them were resistant to ampicillin and one was resistant to gentamicin. The vancomycin resistant strain, also resistant to 6 antibiotics in total was susceptible to ampicillin and gentamicin.

In *E. faecium*, where more strains were tested, less than 5% of the strains remained fully susceptible, mainly caused by resistance to quinupristin/dalfopristin. Fifty percent of the strains were resistant to at least two antibiotics. Two strains were resistant to as much as 7 antibiotics. All of them were vancomycin and ciprofloxacin susceptible, but one of them were resistant to both ampicillin and gentamicin and one was resistant to linezolid.

3.4 Bovines

In 2013, only 20 *E. faecalis* and 54 *E. faecium* strains were tested.

In *E. faecalis*, no resistance was found against ampicillin, ciprofloxacin, florfenicol linezolid, quinupristin/dalfopristin and vancomycin, while last year the only antibiotic against which no resistance was found was salinomycin. This may be explained due to the low number of antibiotics tested rather than an increased susceptibility. Similarly to poultry and pigs, resistance against tetracycline (57,9%), erythromycin (47,4%) and streptomycin (63,2%) is highest. However, the high number of chloramphenicol is striking, nearly half of the strains was resistant to chloramphenicol which is extremely high for an antibiotic not used anymore since almost 20 years. The only other antibiotic against which resistance was found was gentamicin, a bit more than 5% of the strains was resistant.

In *E. faecium*, against all antibiotics, but chloramphenicol, resistance was found. Striking is the absence of resistance in this species, while it is present at relatively high numbers in *E. faecalis* from the same animal population. Similarly as in the other enterococcal populations, the highest resistances were seen against tetracycline, erythromycin, and streptomycin, though lower than for *E. faecalis* (not significant, due to low



numbers of tested strains). Highest resistance was seen against quinupristin/dalfopristin, with 82.7% of the strains being resistant.

In *E. faecalis*, approximately a quarter of the strains was susceptible to all antibiotics tested. Approximately fifty% of the strains were resistant to two or more antibiotics. One strain was resistant to as much as 5 antibiotics but this did not include the clinically important antibiotics ampicillin and vancomycin, but included gentamicin.

In *E. faecium*, 7.7% of the strains remained susceptible to all antibiotics. Most strains were only resistant to one antibiotic in most cases quinupristin/dalfopristin. A bit less than 20% of the strains was resistant to two or more antibiotics. Three strains were resistant to 6 antibiotics and two to 5 antibiotics. One strain was resistant to ampicillin and gentamicin next to ciprofloxacin, but remained susceptible to vancomycin and linezolid. The linezolid resistant strain remained susceptible to the clinically important antibiotics.

3.5 Comparison between animal species

Striking is the similarity that for each origin (animal species) and for each enterococcal species tested, the highest resistances were against tetracycline erythromycin and streptomycin. For the species in the *E. faecium* group, also quinupristin/dalfopristin should be taken into account. In poultry and veal calves, there is in general more resistance against the antibiotics tested compared to the other animal species, though care should be taken since for some combinations, very few strains were available resulting in large confidence intervals not allowing to make firm conclusions.

Resistance to chloramphenicol, an antibiotic not being used anymore for nearly 20 years is still present and especially in *E. faecalis*, and to a much lesser extend in *E. faecium*. This was especially true for strains from veal calves and bovines for which it about half of the strains are resistant. Chloramphenicol resistance can be mediated by a gene encoding a chloramphenicol acetyl transferase, which does not give cross resistance with florfenicol. At the other hand there is the *fex* gene that has recently been described in *Staphylococcus* spp. but not yet in enterococci. It may be that this resistance gene has now been introduced in both *E. faecalis* and *E. faecium* is spreading. Further research is necessary to confirm this.

Ampicillin resistance is mainly associated with *E. faecium* and this mainly in poultry, followed by bovines. This type of resistance is chromosomally mediated, and a large part of its spread might be clonal. Ampicillin resistance is a major therapeutic antibiotic in the treatment of human enterococcal infections.

Vancomycin resistance has been detected in all animal species tested, however, not always in the same bacterial species. Percentage of resistant strains remains however low. Striking is that most strains have only a low level of resistance. Only one strain had a high level of resistance. Further investigations into the meaning of this low level resistance are necessary.



4 Conclusions

This is the third and last large nationwide study on antimicrobial resistance in enterococci in Belgium. Unfortunately also this year, as in the first year, the number of strains was far from ideal for most of the samples to have an accurate estimation of the resistance prevalence.

Marked differences were seen between resistance in *E. faecalis* and *E. faecium* concerning resistance to quinupristin/dalfopristin, a streptogramin antibiotic. Normal MICs of *E. faecalis* to this antibiotic is much higher compared to *E. faecium*. Moreover, there is partial cross-resistance of this antibiotic with the antibiotics of the macrolide-lincosamide-streptogramin (MLS) group of antibiotics. This may explain the high levels of resistance seen in *E. faecium*, since most strains were resistant to erythromycin, a macrolide antibiotic. Erythromycin resistance is caused mostly by the presence of *erm* genes, encoding a methylase of the ribosomal RNA. Since macrolides and streptogramins have overlapping binding sites, this affects the susceptibility of the bacterium. Streptogramins however are always composed of a streptogramin A and a streptogramin B compound. The cross-resistance affects the binding of the B compound to the RNA, while the A compound remains active, resulting in a discrete increase of MICs, as seen in most of the strains in this surveillance. There exist however also specific resistance genes for streptogramins causing a high level of resistance. These high MICs are however more rarely encountered. A molecular investigation towards the genetic background of the resistance is the only way to determine the difference (“breakpoint”) between the high and low level resistances.

Ampicillin resistance was mainly associated with *E. faecium*, while the prevalence of this resistance in *E. faecalis* was much lower. Gentamicin and vancomycin resistance remains low.

Concerning multi-resistance, *E. faecium* was in general more multi-resistant than *E. faecalis*, though this is mainly on the account of quinupristin/dalfopristin resistance. With the exception of *E. faecalis* from pigs and veal calves, less than 10% of the strains were fully susceptible. Highly multi-resistant strains were only a small fraction of the strains.

Except for *E. faecalis* from pigs, few strains remained fully susceptible.



Table 1. List of abbreviations

Abbreviation	
AMP	Ampicillin
CHL	Chloramphenicol
CIP	Ciprofloxacin
ERY	Erythromycin
FFN	Florfenicol
GEN	Gentamicin
LIN	Linezolid
SAL	Salinomycin
STr	Streptomycin
SYN	Synercid (quinupristin/dalfopristin)
TET	Tetracycline
VAN	Vancomycin



Table 2. Antibiotic resistance in commensal *Enterococci* from poultry.

		AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<i>E. faecalis</i>	N	71	71	71	71	71	71	71	71	71	71	71	71
	NR	4	3	1	50	1	0	0	8	36	2	63	0
	%R	5,6	4,2	1,4	70,4	1,4	0,0	0,0	11,3	50,7	2,8	88,7	0,0
	CI	2,1-14,4	1,3-12,6	0,2-9,7	58,5-80,0	0,2-9,7	0-5,0	0-5,0	5,6-21,2	39,0-62,4	0,7-10,9	78,7-94,4	0-5,0
<i>E. faecium</i>	N	113	113	113	113	113	113	113	113	113	113	113	113
	NR	43	0	10	85	0	1	0	61	63	100	76	1
	%R	38,1	0,0	8,8	75,2	0,0	0,9	0,0	54,0	55,8	88,5	67,3	0,9
	CI	29,5-47,5	0-3,2	4,8-15,8	66,3-82,4	0-3,2	0,1-6,2	0-3,2	44,6-63,1	46,4-64,8	81,1-93,3	58,0-75,4	0,1-6,2



Tabel 3. Antibiotic resistance in commensal *Enterococci* from pigs.

		AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<i>E. faecalis</i>	N	13	13	13	13	13	13	13	13	13	13	13	13
	NR	0	3	0	4	0	0	0	0	3	0	7	0
	%R	0,0	23,1	0,0	30,8	0,0	0,0	0,0	0,0	23,1	0,0	53,8	0,0
	CI	0-24,7	6,3-57,2	0-24,7	10,2-63,5	0-24,7	0-24,7	0-24,7	0-24,7	6,3-57,2	0-24,7	24,9-80,5	0-24,7
<i>E. faecium</i>	N	69	69	69	69	69	69	69	69	69	69	69	69
	NR	6	0	4	14	0	1	1	4	10	60	19	1
	%R	8,7	0,0	5,8	20,3	0,0	1,4	1,4	5,8	14,5	87,0	27,5	1,4
	CI	3,9-18,4	0-5,2	2,1-14,8	12,2-31,7	0-5,2	0,2-10,0	0,2-10,0	2,1-14,8	7,9-25,2	76,5-93,2	18,1-39,5	0,2-10,0



Table 4 Antibiotic resistance in commensal *Enterococci* from veal calves.

		AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<i>E. faecalis</i>	N	46	46	46	46	46	46	46	46	46	46	46	46
	NR	3	29	2	40	2	6	0	2	32	2	42	1
	%R	6,5	63,0	4,3	87,0	4,3	13,0	0,0	4,3	69,6	4,3	91,3	2,2
	CI	2,0-19,5	47,8-76,1	1,0-16,5	73,2-94,2	1,0-16,5	5,8-26,8	0-7,7	1,0-16,5	54,3-81,5	1,0-16,5	78,3-96,8	0,3-14,8
<i>E. faecium</i>	N	107	107	107	107	107	107	107	107	107	107	107	107
	NR	19	4	2	56	5	3	2	2	42	96	55	3
	%R	17,8	3,7	1,9	52,3	4,7	2,8	1,9	1,9	39,3	89,7	51,4	2,8
	CI	11,5-26,3	1,4-9,7	0,5-7,3	42,7-61,7	1,9-10,9	0,9-8,5	0,5-7,3	0,5-7,3	30,3-48,9	82,2-94,3	41,8-60,9	0,9-8,5



Table 5. Antibiotic resistance in commensal *Enterococci* from bovines.

		AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<i>E. faecalis</i>	N	20	20	20	20	20	20	20	20	20	20	20	20
	NR	0	9	0	10	0	1	0	1	12	0	12	0
	%R	0,0	45,0	0,0	50,0	0,0	5,0	0,0	5,0	60,0	0,0	60,0	0,0
	CI	0-16,8	23,8-68,2	0-16,8	27,7-72,3	0-16,8	0,6-32,3	0-16,8	0,6-32,3	36,0-80,0	0-16,8	36,0-80,0	0-16,8
<i>E. faecium</i>	N	54	54	54	54	54	54	54	54	54	54	54	54
	NR	2	0	4	11	2	1	1	4	7	46	9	1
	%R	3,7	0,0	7,4	20,4	3,7	1,9	1,9	7,4	13,0	85,2	16,7	1,9
	CI	0,9-14,2	0-6,6	2,7-18,6	11,4-33,6	0,9-14,2	0,2-12,7	0,2-12,7	2,7-18,6	6,2-25,3	72,6-92,6	8,7-29,5	0,2-12,7



Table 6. Antimicrobial resistance in in *E. faecalis* from different animal species.

		AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
Bovines	N	20	20	20	20	20	20	20	20	20	20	20	20
	NR	0	9	0	10	0	1	0	1	12	0	12	0
	%R	0,0	45,0	0,0	50,0	0,0	5,0	0,0	5,0	60,0	0,0	60,0	0,0
	CI	0-16,8	23,8-68,2	0-16,8	27,7-72,3	0-16,8	0,6-32,3	0-16,8	0,6-32,3	36,0-80,0	0-16,8	36,0-80,0	0-16,8
Veal calves	N	46	46	46	46	46	46	46	46	46	46	46	46
	NR	3	29	2	40	2	6	0	2	32	2	42	1
	%R	6,5	63,0	4,3	87,0	4,3	13,0	0,0	4,3	69,6	4,3	91,3	2,2
	CI	2,0-19,5	47,8-76,1	1,0-16,5	73,2-94,2	1,0-16,5	5,8-26,8	0-7,7	1,0-16,5	54,3-81,5	1,0-16,5	78,3-96,8	0,3-14,8
Poultry	N	71	71	71	71	71	71	71	71	71	71	71	71
	NR	4	3	1	50	1	0	0	8	36	2	63	0
	%R	5,6	4,2	1,4	70,4	1,4	0,0	0,0	11,3	50,7	2,8	88,7	0,0
	CI	2,1-14,4	1,3-12,6	0,2-9,7	58,5-80,0	0,2-9,7	0-5,0	0-5,0	5,6-21,2	39,0-62,4	0,7-10,9	78,7-94,4	0-5,0
Pig	N	13	13	13	13	13	13	13	13	13	13	13	13
	NR	0	3	0	4	0	0	0	0	3	0	7	0
	%R	0,0	23,1	0,0	30,8	0,0	0,0	0,0	0,0	23,1	0,0	53,8	0,0
	CI	0-24,7	6,3-57,2	0-24,7	10,2-63,5	0-24,7	0-24,7	0-24,7	0-24,7	6,3-57,2	0-24,7	24,9-80,5	0-24,7



Table 7. Antimicrobial resistance in in *E. faecium* from different animal species.

		AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
Bovines	N	54	54	54	54	54	54	54	54	54	54	54	54
	NR	2	0	4	11	2	1	1	4	7	46	9	1
	%R	3,7	0,0	7,4	20,4	3,7	1,9	1,9	7,4	13,0	85,2	16,7	1,9
	CI	0,9-14,2	0-6,6	2,7-18,6	11,4-33,6	0,9-14,2	0,2-12,7	0,2-12,7	2,7-18,6	6,2-25,3	72,6-92,6	8,7-29,5	0,2-12,7
Veal calves	N	107	107	107	107	107	107	107	107	107	107	107	107
	NR	19	4	2	56	5	3	2	2	42	96	55	3
	%R	17,8	3,7	1,9	52,3	4,7	2,8	1,9	1,9	39,3	89,7	51,4	2,8
	CI	11,5-26,3	1,4-9,7	0,5-7,3	42,7-61,7	1,9-10,9	0,9-8,5	0,5-7,3	0,5-7,3	30,3-48,9	82,2-94,3	41,8-60,9	0,9-8,5
Poultry	N	113	113	113	113	113	113	113	113	113	113	113	113
	NR	43	0	10	85	0	1	0	61	63	100	76	1
	%R	38,1	0,0	8,8	75,2	0,0	0,9	0,0	54,0	55,8	88,5	67,3	0,9
	CI	29,5-47,5	0-3,2	4,8-15,8	66,3-82,4	0-3,2	0,1-6,2	0-3,2	44,6-63,1	46,4-64,8	81,1-93,3	58,0-75,4	0,1-6,2
Pig	N	69	69	69	69	69	69	69	69	69	69	69	69
	NR	6	0	4	14	0	1	1	4	10	60	19	1
	%R	8,7	0,0	5,8	20,3	0,0	1,4	1,4	5,8	14,5	87,0	27,5	1,4
	CI	3,9-18,4	0-5,2	2,1-14,8	12,2-31,7	0-5,2	0,2-10,0	0,2-10,0	2,1-14,8	7,9-25,2	76,5-93,2	18,1-39,5	0,2-10,0



Table 10 Antimicrobial resistance in *E. faecalis* from poultry

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	8	0	0	0	1	5	0	0	7	0
1	51	0	51	14	1	0	22	26	0	0	1	41
2	12	0	7	6	4	0	48	6	0	2	0	25
4	4	3	4	1	65	0	0	26	0	0	0	5
8	1	57	1	0	0	20	0	8	0	14	0	0
16	0	5	0	0	1	51	0	0	0	50	0	0
32	1	3	0	0	0	0	0	0	0	3	12	0
64	1	3	0	2	0	0	0	0	3	2	18	0
128	0	0	0	1	0	0	0	0	30	0	33	0
256	1	0	0	47	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0	2	0	0	0
1024	0	0	0	0	0	0	0	0	1	0	0	0
>1024	0	0	0	0	0	0	0	0	35	0	0	0
N	71	71	71	71	71	71	71	71	71	71	71	71
NR	4	3	1	50	1	0	0	8	36	2	63	0
%R	5,6	4,2	1,4	70,4	1,4	0,0	0,0	11,3	50,7	2,8	88,7	0,0
CI	2,1-14,4	1,3-12,6	0,2-9,7	80,0	0,2-9,7	0-5,0	0-5,0	5,6-21,2	62,4	0,7-10,9	94,4	0-5,0



Table 11 Antimicrobial resistance in *E. faecium* from poultry

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	4	0	0	0	0	0	0	3	35	31
1	31	0	21	4	0	0	6	11	0	10	1	64
2	27	0	35	12	4	0	105	18	0	6	1	14
4	12	7	43	12	107	3	2	23	0	34	4	3
8	24	86	10	3	2	73	0	61	0	51	0	1
16	1	7	0	0	0	35	0	0	0	4	0	0
32	2	13	0	0	0	1	0	0	2	5	0	0
64	3	0	0	0	0	0	0	0	41	0	13	0
128	11	0	0	0	0	0	0	0	7	0	59	0
256	2	0	0	82	0	0	0	0	1	0	0	0
512	0	0	0	0	0	0	0	0	3	0	0	0
1024	0	0	0	0	0	1	0	0	9	0	0	0
>1024	0	0	0	0	0	0	0	0	50	0	0	0
N	113	113	113	113	113	113	113	113	113	113	113	113
NR	43	0	10	85	0	1	0	61	63	100	76	1
%R	38,1	0,0	8,8	75,2	0,0	0,9	0,0	54,0	55,8	88,5	67,3	0,9
CI	29,5-47,5	0-3,2	4,8-15,8	66,3-82,4	0-3,2	0,1-6,2	0-3,2	44,6-63,1	46,4-64,8	81,1-93,3	58,0-75,4	0,1-6,2



Table 14 Antimicrobial resistance in *E. faecalis* from pigs.

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	2	0	0	0	1	2	0	0	4	0
1	11	0	7	6	1	0	0	10	0	0	2	9
2	1	0	4	2	3	0	12	0	0	0	0	3
4	1	0	0	1	9	0	0	1	0	0	0	1
8	0	9	0	0	0	6	0	0	0	3	0	0
16	0	0	0	0	0	5	0	0	0	9	0	0
32	0	1	0	0	0	2	0	0	0	1	0	0
64	0	1	0	0	0	0	0	0	1	0	3	0
128	0	2	0	0	0	0	0	0	9	0	4	0
256	0	0	0	4	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	0	0	0
>1024	0	0	0	0	0	0	0	0	3	0	0	0
N	13	13	13	13	13	13	13	13	13	13	13	13
NR	0	3	0	4	0	0	0	0	3	0	7	0
%R	0,0	23,1	0,0	30,8	0,0	0,0	0,0	0,0	23,1	0,0	53,8	0,0
CI	0-24,7	6,3-57,2	0-24,7	10,2-63,5	0-24,7	0-24,7	0-24,7	0-24,7	6,3-57,2	0-24,7	80,5-24,9	0-24,7



Table 15. Antimicrobial resistance in *E. faecium* from pigs.

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	11	0	0	0	0	0	0	2	47	33
1	32	0	38	4	0	0	0	23	0	7	2	30
2	19	0	10	26	5	0	68	38	0	3	1	2
4	12	11	6	25	63	2	0	4	0	52	0	3
8	6	57	4	2	1	46	0	3	0	4	2	1
16	0	0	0	1	0	17	0	0	0	0	0	0
32	0	1	0	1	0	3	1	0	1	0	0	0
64	0	0	0	2	0	0	0	0	53	1	12	0
128	0	0	0	0	0	0	0	1	5	0	5	0
256	0	0	0	8	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	1	0	0	3	0	0	0
>1024	0	0	0	0	0	0	0	0	7	0	0	0
N	69	69	69	69	69	69	69	69	69	69	69	69
NR	6	0	4	14	0	1	1	4	10	60	19	1
%R	8,7	0,0	5,8	20,3	0,0	1,4	1,4	5,8	14,5	87,0	27,5	1,4
CI	3,9-18,4	0-5,2	2,1-14,8	12,2-31,7	0-5,2	0,2-10,0	0,2-10,0	2,1-14,8	7,9-25,2	93,2	18,1-39,5	0,2-10,0



Table 18. Antimicrobial resistance in *E. faecalis* from veal calves.

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	15	0	0	0	2	12	0	0	2	1
1	31	0	25	3	3	0	16	30	0	0	2	27
2	11	0	3	3	11	0	28	1	0	1	0	13
4	1	3	1	0	30	0	0	1	0	10	0	4
8	0	12	0	0	0	26	0	2	0	6	0	1
16	0	1	1	0	1	13	0	0	0	24	1	0
32	2	1	0	0	1	1	0	0	1	3	0	0
64	0	25	1	0	0	1	0	0	6	2	5	0
128	0	4	0	0	0	0	0	0	6	0	36	0
256	1	0	0	40	0	0	0	0	1	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	5	0	0	1	0	0	0
>1024	0	0	0	0	0	0	0	0	31	0	0	0
N	46	46	46	46	46	46	46	46	46	46	46	46
NR	3	29	2	40	2	6	0	2	32	2	42	1
%R	6,5	63,0	4,3	87,0	4,3	13,0	0,0	4,3	69,6	4,3	91,3	2,2
CI	2,0-19,5	47,8-76,1	1,0-16,5	73,2-94,2	1,0-16,5	5,8-26,8	0-7,7	1,0-16,5	81,5	1,0-16,5	96,8	0,3-14,8



Table 19. Antimicrobial resistance in *E. faecium* from veal calves.

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	1	0	0	0	0	0
0.5	0	0	10	0	0	0	0	1	0	1	47	32
1	48	0	42	2	1	0	11	45	0	10	3	51
2	30	0	25	15	8	0	92	57	0	4	2	16
4	10	10	28	34	93	11	1	2	0	63	0	5
8	16	79	1	8	0	61	1	1	0	24	2	2
16	0	5	1	0	0	29	0	0	2	2	1	0
32	1	9	0	1	3	3	0	0	6	2	0	0
64	0	4	0	1	1	0	1	0	51	1	3	0
128	2	0	0	0	1	0	0	1	6	0	49	1
256	0	0	0	46	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0	1	0	0	0
1024	0	0	0	0	0	3	0	0	2	0	0	0
>1024	0	0	0	0	0	0	0	0	39	0	0	0
N	107	107	107	107	107	107	107	107	107	107	107	107
NR	19	4	2	56	5	3	2	2	42	96	55	3
%R	17,8	3,7	1,9	52,3	4,7	2,8	1,9	1,9	39,3	89,7	51,4	2,8
CI	11,5-26,3	1,4-9,7	0,5-7,3	42,7-61,7	1,9-10,9	0,9-8,5	0,5-7,3	0,5-7,3	30,3-48,9	82,2-94,3	41,8-60,9	0,9-8,5



Table 22. Antimicrobial resistance in *E. faecalis* from bovines.

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	4	0	0	0	0	3	0	1	5	2
1	17	0	12	8	0	0	6	16	0	0	3	13
2	2	0	3	2	5	0	14	0	0	0	0	2
4	1	2	1	0	15	0	0	0	0	3	0	3
8	0	7	0	0	0	9	0	1	0	8	0	0
16	0	1	0	0	0	10	0	0	0	7	1	0
32	0	1	0	0	0	0	0	0	0	1	0	0
64	0	8	0	0	0	0	0	0	2	0	5	0
128	0	1	0	0	0	0	0	0	5	0	6	0
256	0	0	0	10	0	0	0	0	1	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	1	0	0	0	0	0	0
>1024	0	0	0	0	0	0	0	0	12	0	0	0
N	20	20	20	20	20	20	20	20	20	20	20	20
NR	0	9	0	10	0	1	0	1	12	0	12	0
%R	0,0	45,0	0,0	50,0	0,0	5,0	0,0	5,0	60,0	0,0	60,0	0,0
CI	0-16,8	23,8-68,2	0-16,8	27,7-72,3	0-16,8	0,6-32,3	0-16,8	0,6-32,3	36,0-80,0	0-16,8	36,0-80,0	0-16,8



Table 23. Antimicrobial resistance in *E. faecium* from bovines.

Concentration	AMP	CHL	CIP	ERY	FFN	GEN	LZD	SAL	Str	SYN	TET	VAN
<=0.25	0	0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	5	0	0	0	0	0	0	0	45	13
1	31	0	30	6	0	0	0	14	0	8	0	33
2	18	0	8	12	2	0	48	34	0	4	0	4
4	3	9	7	25	50	2	5	2	0	39	0	3
8	1	39	4	2	0	27	1	4	0	2	0	1
16	0	2	0	0	0	19	0	0	1	1	0	0
32	0	4	0	0	0	5	0	0	4	0	2	0
64	0	0	0	0	1	0	0	0	40	0	1	0
128	1	0	0	0	1	0	0	0	2	0	6	0
256	0	0	0	9	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	1	0	0	0	0	0	0
>1024	0	0	0	0	0	0	0	0	7	0	0	0
N	54	54	54	54	54	54	54	54	54	54	54	54
NR	2	0	4	11	2	1	1	4	7	46	9	1
%R	3,7	0,0	7,4	20,4	3,7	1,9	1,9	7,4	13,0	85,2	16,7	1,9
CI	0,9-14,2	0-6,6	2,7-18,6	11,4-33,6	0,9-14,2	0,2-12,7	0,2-12,7	2,7-18,6	6,2-25,3	72,6-92,6	8,7-29,5	0,2-12,7



Table 26. Multi-resistance in *E. faecalis* from poultry.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	8	11,3	11,3
1	13	18,3	29,6
2	9	12,7	42,3
3	31	43,7	85,9
4	7	9,9	95,8
5	2	2,8	98,6
6	1	1,4	100,0
7	0	0,0	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 1. Multi-resistance in *E. faecalis* from poultry expressed as percentage of strains having resistance to N antibiotics

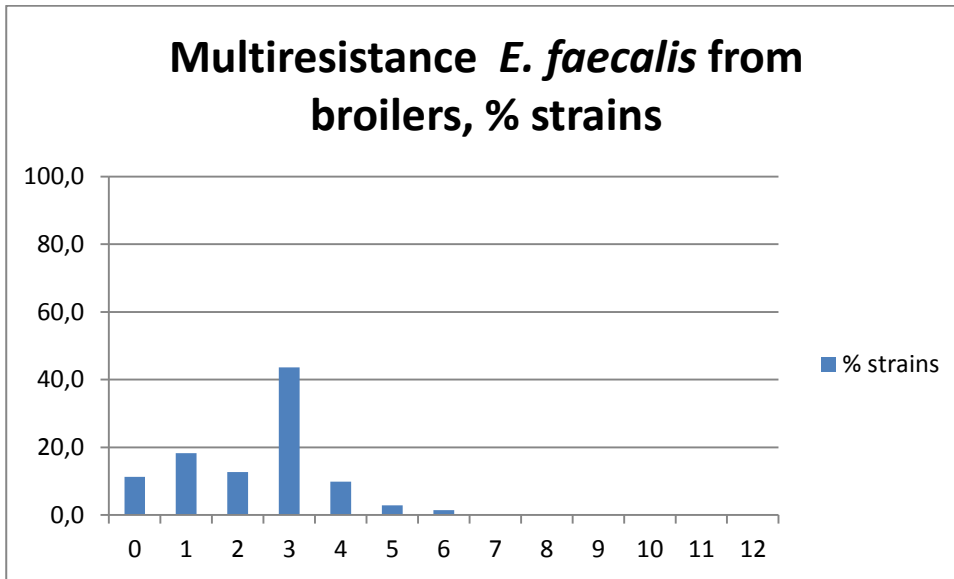


Figure 2. Multi-resistance in *E. faecalis* from poultry expressed as cumulative percentage of strains having resistance to N antibiotics

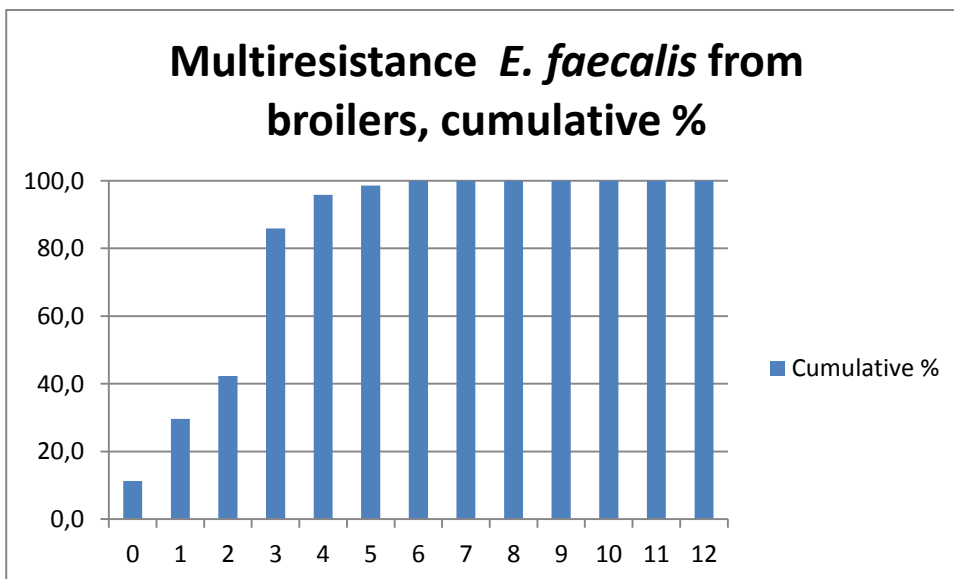




Table 27. Multi-resistance in *E. faecium* from poultry.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	4	3,5	3,5
1	24	21,2	24,8
2	15	13,3	38,1
3	18	15,9	54,0
4	17	15,0	69,0
5	25	22,1	91,2
6	9	8,0	99,1
7	1	0,9	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 3. Multi-resistance in *E. faecium* from poultry expressed as percentage of strains having resistance to N antibiotics

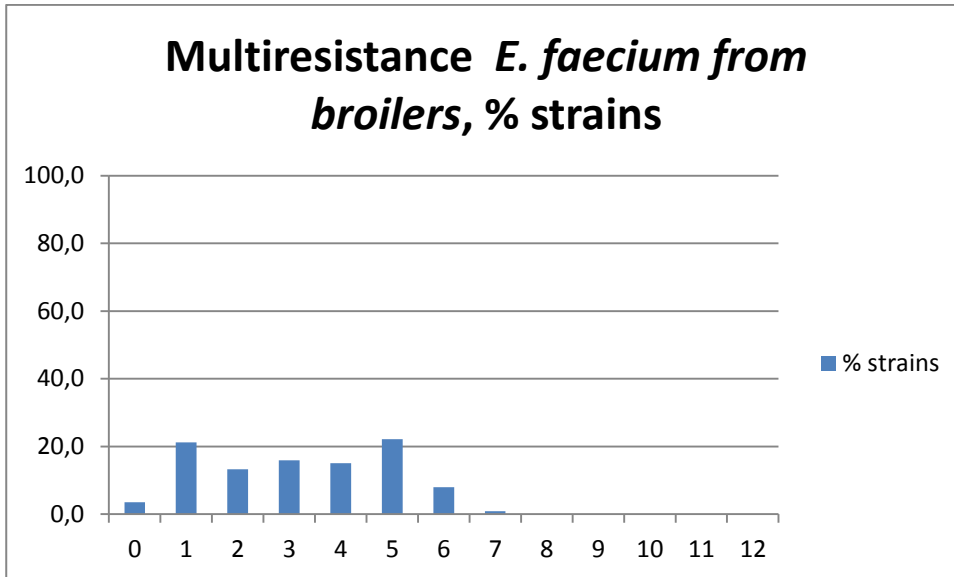


Figure 4. Multi-resistance in *E. faecium* from poultry expressed as cumulative percentage of strains having resistance to N antibiotics

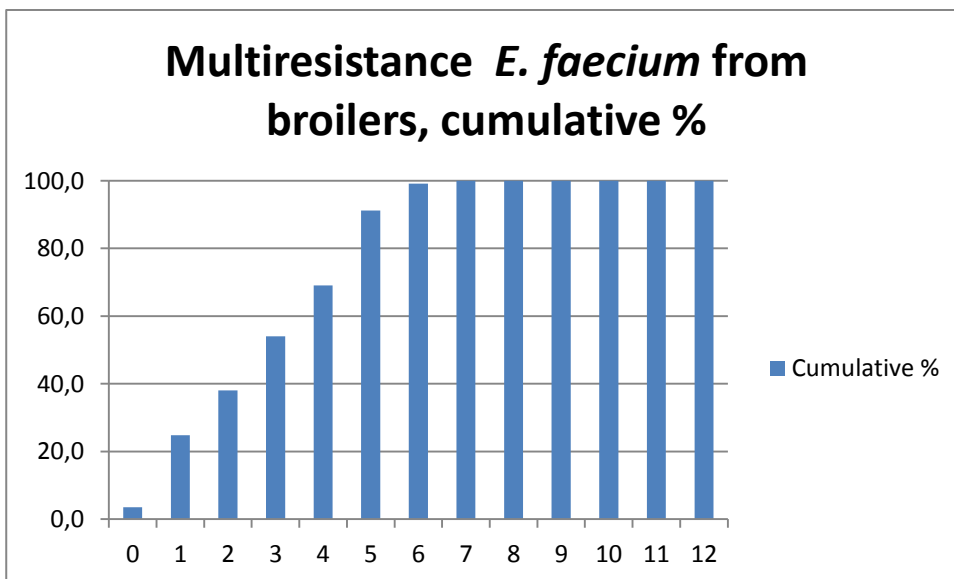




Table 30. Multi-resistance in *E. faecalis* from pigs.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	6	46,2	46,2
1	3	23,1	69,2
2	0	0,0	69,2
3	2	15,4	84,6
4	2	15,4	100,0
5	0	0,0	100,0
6	0	0,0	100,0
7	0	0,0	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 9. Multi-resistance in *E. faecalis* from pigs expressed as percentage of strains having resistance to N antibiotics.

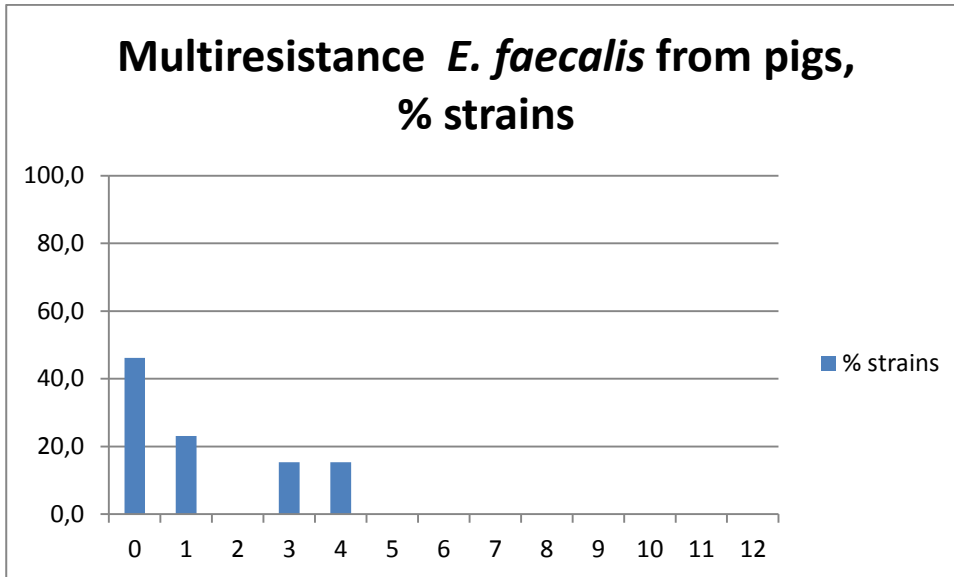


Figure 10. Multi-resistance in *E. faecalis* from pigs expressed as cumulative percentage of strains having resistance to N antibiotics

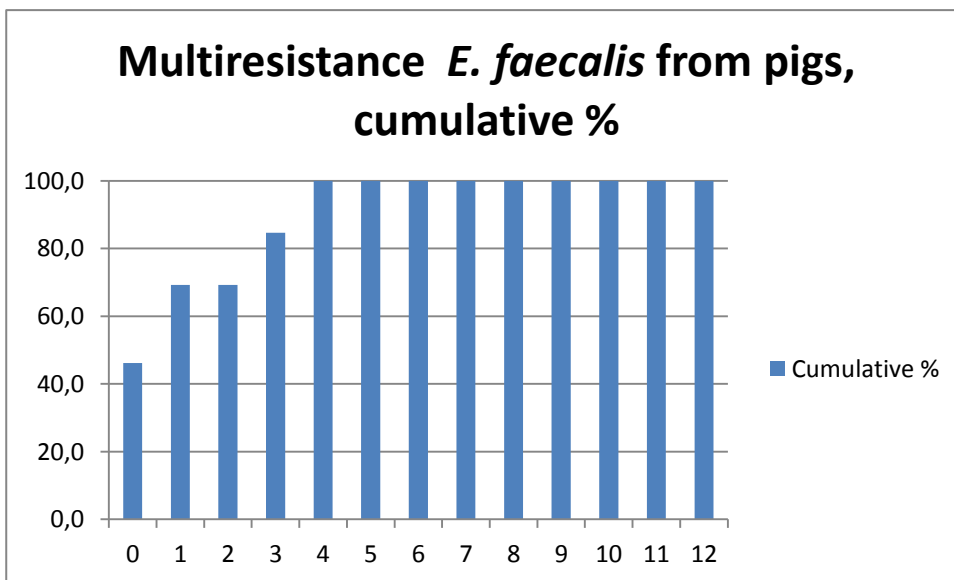




Table 31. Multi-resistance in *E. faecium* from pigs.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	2	2,9	2,9
1	19	27,5	30,4
2	20	29,0	59,4
3	10	14,5	73,9
4	9	13,0	87,0
5	6	8,7	95,7
6	3	4,3	100,0
7	0	0,0	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0
12	0	0,0	100,0



Figure 11. Multi-resistance in *E. faecium* from pigs expressed as percentage of strains having resistance to N antibiotics.

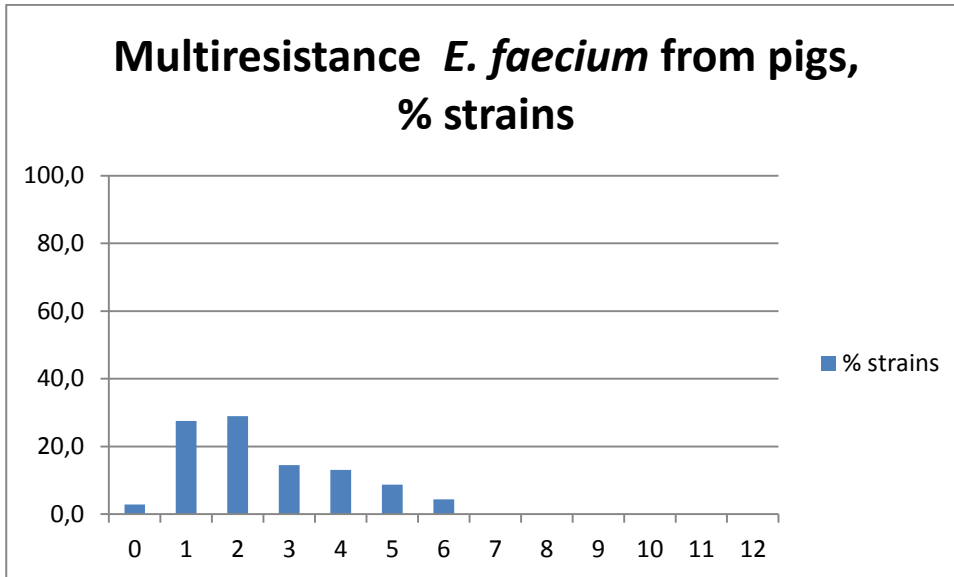


Figure 12. Multi-resistance in *E. faecium* from pigs expressed as cumulative percentage of strains having resistance to N antibiotics

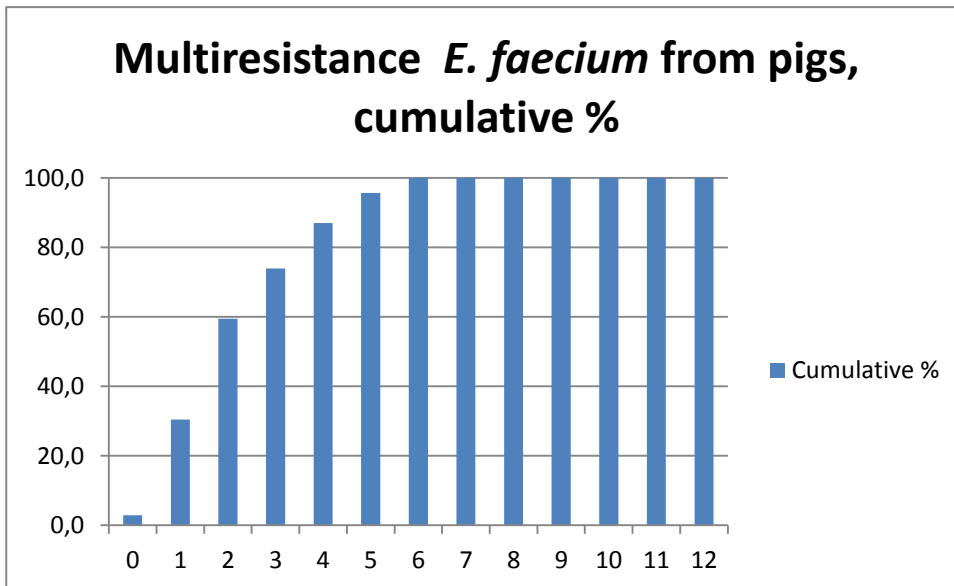




Table 34. Multi-resistance in *E. faecalis* from veal calves.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	4	8,7	8,7
1	1	2,2	10,9
2	4	8,7	19,6
3	8	17,4	37,0
4	21	45,7	82,6
5	4	8,7	91,3
6	4	8,7	100,0
7	0	0,0	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 17. Multi-resistance in *E. faecalis* from veal calves expressed as percentage of strains having resistance to N antibiotics.

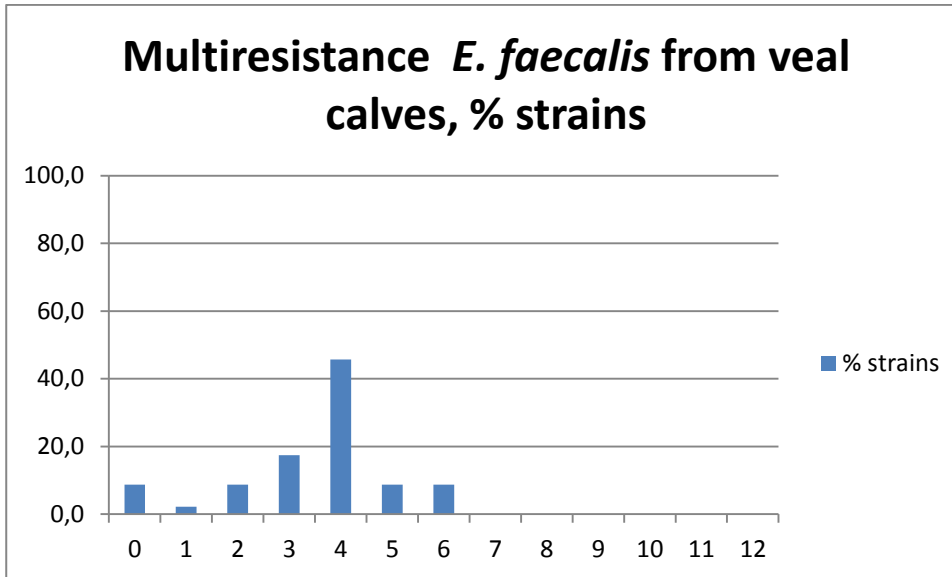


Figure 18. Multi-resistance in *E. faecalis* from veal calves expressed as cumulative percentage of strains having resistance to N antibiotics

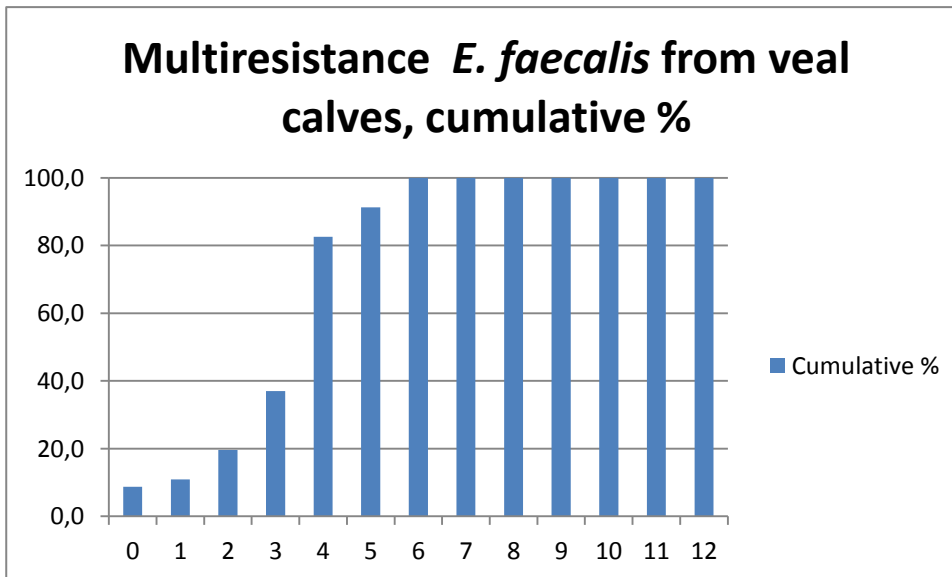




Table 35. Multi-resistance in *E. faecium* from veal calves.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	4	3,7	3,7
1	19	17,8	21,5
2	31	29,0	50,5
3	22	20,6	71,0
4	14	13,1	84,1
5	11	10,3	94,4
6	4	3,7	98,1
7	2	1,9	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 19. Multi-resistance in *E. faecium* from veal calves expressed as percentage of strains having resistance to N antibiotics

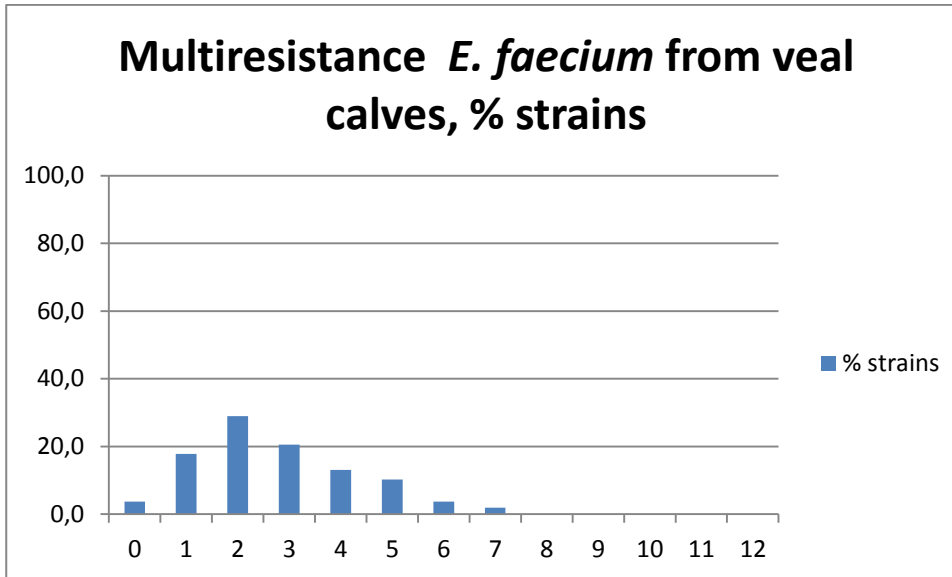


Figure 20. Multi-resistance in *E. faecium* from veal calves expressed as cumulative percentage of strains having resistance to N antibiotics

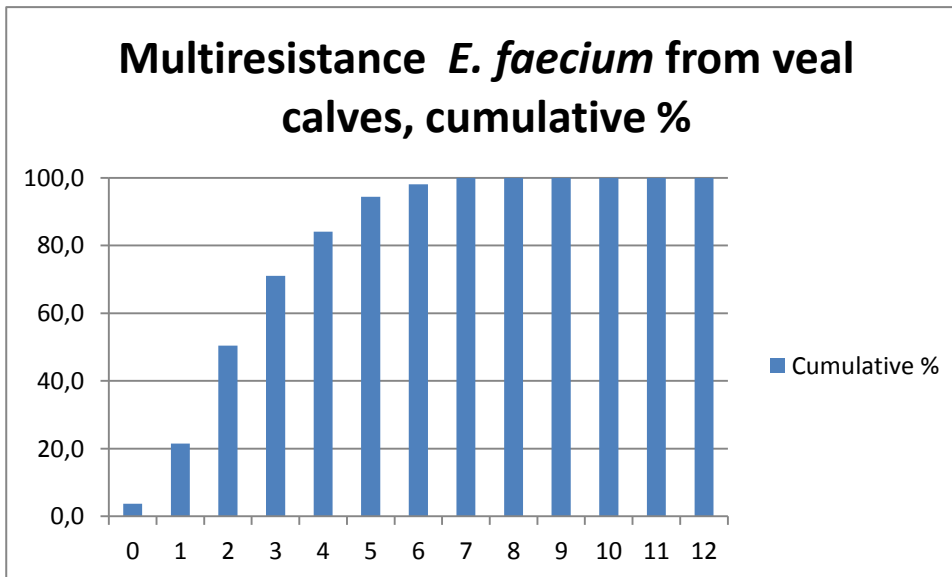




Table 38. Multi-resistance in *E. faecalis* from bovines.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	5	25,0	25,0
1	2	10,0	35,0
2	2	10,0	45,0
3	6	30,0	75,0
4	4	20,0	95,0
5	1	5,0	100,0
6	0	0,0	100,0
7	0	0,0	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 25. Multi-resistance in *E. faecalis* from bovines expressed as percentage of strains having resistance to N antibiotics.

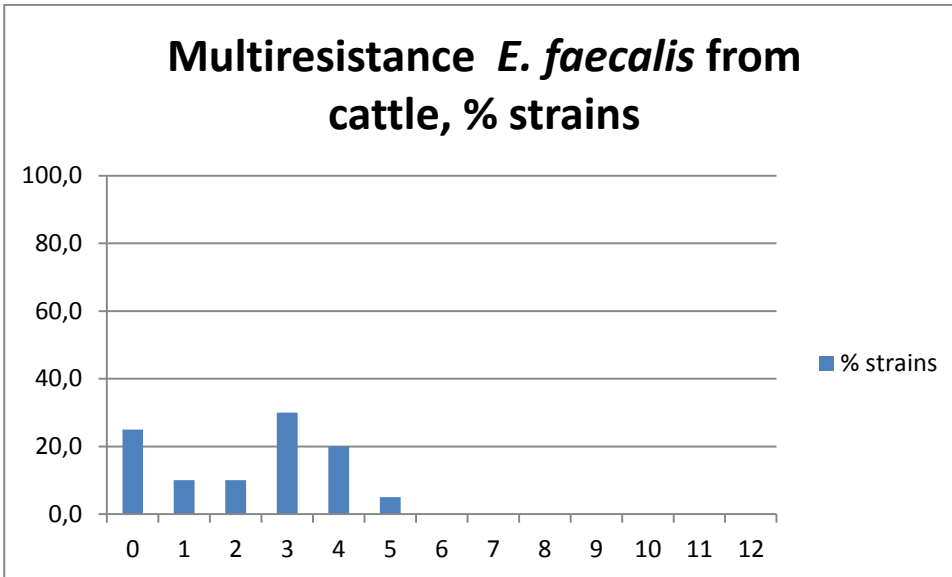


Figure 26. Multi-resistance in *E. faecalis* from bovines expressed as cumulative percentage of strains having resistance to N antibiotics

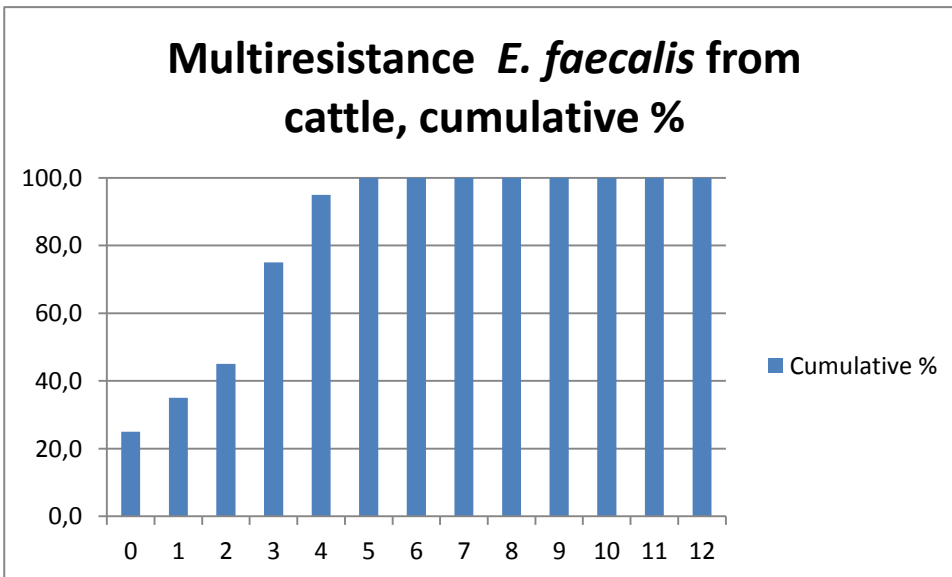




Table 39. Multi-resistance in *E. faecium* from bovines.

Number of antimicrobials	Number of strains	% of bacteria	Cumulative %
0	4	7,5	7,5
1	26	49,1	56,6
2	10	18,9	75,5
3	5	9,4	84,9
4	3	5,7	90,6
5	4	7,5	98,1
6	1	1,9	100,0
7	0	0,0	100,0
8	0	0,0	100,0
9	0	0,0	100,0
10	0	0,0	100,0
11	0	0,0	100,0
12	0	0,0	100,0



Figure 27. Multi-resistance in *E. faecium* from bovines expressed as percentage of strains having resistance to N antibiotics.

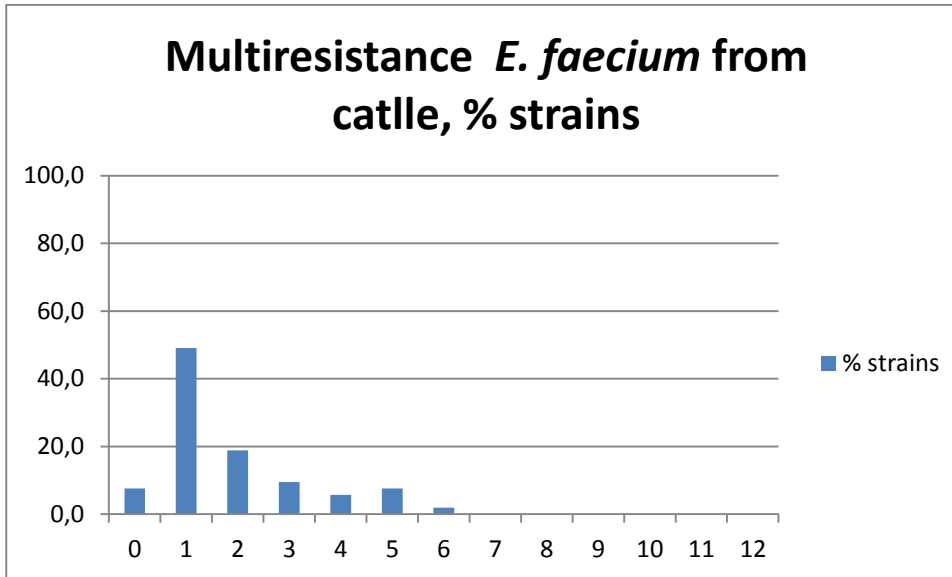


Figure 28. Multi-resistance in *E. faecium* from bovines expressed as cumulative percentage of strains having resistance to N antibiotics

