Antimicrobial Resistance Programme
Specialist Antimicrobial Chemotherapy Unit

3rd Generation Cephalosporin Resistance in Wales 2008

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Date: 9th September 2010 | **Version:** 1

Publication/ Distribution: (Delete as applicable)

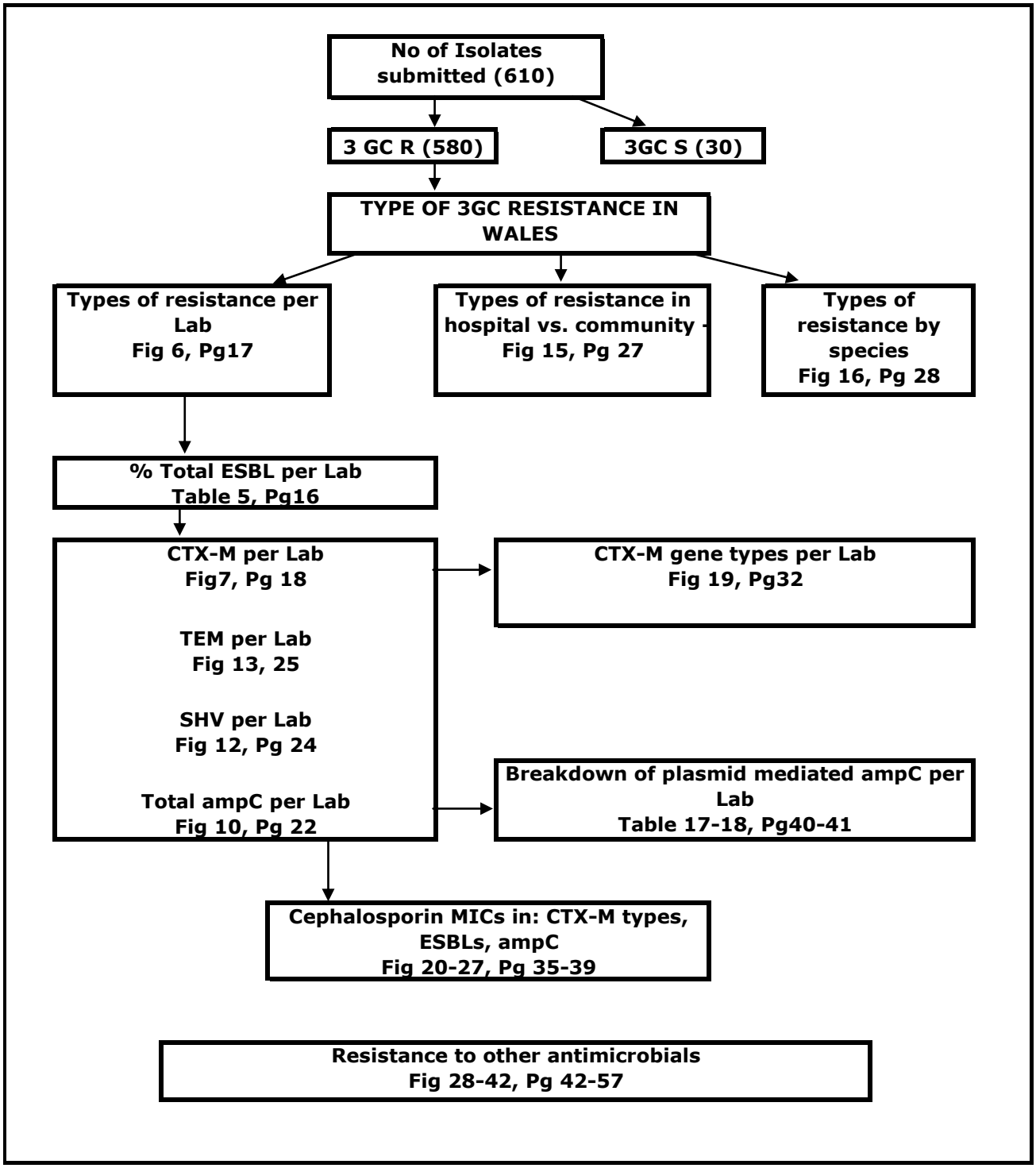
- NHS Wales (Intranet)
- Public Health Wales (Intranet)

Review Date: N/A

Purpose and Summary of Document:

Targeted surveillance to determine prevalence of 3rd generation cephalosporin resistance in Enterobacteriaceae in Wales

Work Plan reference: Perform targeted surveillance


No of Isolates submitted (610)
3 GC R (580)
3GC S (30)
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1 Introduction

Resistance to 3rd generation cephalosporins is an increasing problem, with the emergence and spread of CTX-M and plasmidic ampC-type β-lactamases. In Wales all coliforms are screened for susceptibility to cefpodoxime. In urinary coliforms approximately 6% from the community and 12% from hospitals are resistant to cefpodoxime respectively. This targeted surveillance study will provide an overview of molecular mechanisms responsible for 3rd generation cephalosporin resistance in Wales.

2 Methods

2.1 Strains

In Wales approximately 90,000 hospital and community coliforms are isolated per year, 4500 (5%) of these isolates are cefpodoxime resistant, and of these 3600 (80%) contain extended spectrum β-lactamases (ESBL). Based upon these assumptions and in order to detect any year-on-year increase in resistance, 1/6th of cefpodoxime resistant *Enterobacteriaceae* were requested from Microbiology laboratories across Wales (Table 1). Laboratories asked to participate were: Bronglais (Aberystwyth), Gwynned (Bangor), Prince of Wales (Bridgend), West Wales General (Carmarthen), Nevill Hall (Abergavenny), Glan Clwyd (Rhyl), Royal Glamorgan (Llantrisant), Royal Gwent (Newport), Singleton (Swansea) University Hospital of Wales (Cardiff), Withybush (Haverfordwest) and Maelor (Wrexham). Isolates were divided into *E. coli* and coliforms from Hospital (H) and Community/GP (C).

Table 1: Requested numbers of isolates - 1/6th cefpodoxime resistant coliforms per year per laboratory

Laboratory	Hospital Coliforms	Hospital *E. coli*	GP Coliforms	GP *E. coli*	Totals
Aberystwyth	3	5	4	14	26
Bangor	15	17	16	34	82
Bridgend	11	13	8	19	51
Carmarthen	15	20	11	30	76
Rhyl	17	15	19	30	81
Llantrisant	8	12	4	17	41
Swansea	70	6	68	1	145
Cardiff	24	41	20	62	147

Haverfordwest	11	2	20	3	36
Newport	46	21	29	24	120
Wrexham	7	21	7	32	67
Abergavenny	1	3	3	8	15
Bangor	15	17	16	34	82
Total	243	193	225	308	969

3rd generation cephalosporin resistance was determined in Welsh laboratories using the following methods:

Rhyl	– CPD R (DD), ESBL discs to confirm ESBL
Aberystwyth	– CPD R (DD)
Wrexham	– CPD R (DD)
Abergavenny	– CPD R (DD) + PHX, Combi ESBL discs for confirmation
Carmarthen	– CPD R (DD)
Llantrisant	– CPD R (DD) + PHX, ESBL Etests
Bridgend	– CPD R, then CPD, FOX+CLAV discs
Haverfordwest	– CTX R
Cardiff	– CPD R (DD) + PHX
Newport	– CPD R (DD) + PHX

CPD-R - Cefpodoxime resistant
PHX – Phoenix
DD – disc diffusion
FOX+CLAV – cefoxitin+clavulanate

Upon receipt at SACU, isolates were stored at -80°C on Micro-Bank beads until required.

2.2 Phenotypic Characterisation

2.2.1 Minimum Inhibitory Concentration (MIC) Determination

MICs were determined on IsoSensitest agar according to BSAC standards for ciprofloxacin (Claris Life Sciences Ltd), temocillin (Eumedica), mecillinam (Leo-Pharma), nitrofurantoin (Sigma-Aldrich), gentamicin Sulphate (Sigma-Aldrich), cefoxitin Sigma-Aldrich), cefotaxime (Sigma-Aldrich) and ceftazidime (Sigma-Aldrich), in the range from 0.008 to 128mg/L. Cefoxitin, ceftazidime and cefotaxime

were also tested in combination with either 4mg/L potassium clavulanate (Riedel-de Haën®) or 200mg/L cloxacillin sodium salt monohydrate (Sigma-Aldrich). Inocula were prepared in saline to achieve a 10^8 cfu/ml (0.5 McFarland standard), diluted 1 in 10 then 1µl dispensed by multi-point inoculator to achieve 10^4 cfu/ml per plate. Plates were incubated in air at 35-37°C for 18hrs.

2.2.2 Analysis of Phenotypic Data

MICs were recorded as the lowest antimicrobial concentration where growth of the isolate was inhibited.

The MICs for cefoxitin, cefotaxime and ceftazidime in combination with cloxacillin or clavulanate were compared to predict the presence of Extended Spectrum β-lactamase (ESBL) and/or AmpC enzyme. The following criteria were used:

Extended- spectrum β-lactamase (ESBL) positive if ≥3 fold log2 decrease in MIC in the presence of 4mg/L clavulanate compared to the cephalosporin alone.

AmpC β-lactamase positive if ≥3 fold decrease in MIC in the presence of 200mg/L cloxacillin compared to the cephalosporin alone.

All isolates with resistance to 3rd generation cephalosporin, including ESBL and ampC phenotypes were characterised by genotypic methods.

2.3 Genotypic Characterisation

2.3.1 Polymerase Chain Reaction (PCR)

Isolates were screened for the presence of β-lactamase genes by Polymerase Chain Reaction (PCR) assays using specific primers and annealing temperatures:

bla$_{ctx\text{-}m}$ - annealing temperature of 52°C (Woodford *et al* 2005)

bla$_{tem}$ -annealing at 55°C (Wiegand *et al* JCM 2007)

bla$_{shv}$ - annealing at 49°C (Wiegand *et al* JCM 2007)

Table 2: Primer sequences

Primer name	Forward Sequence	Reverse Sequence
CTX-M gp 1	AAAAATCACTGCGCCAGTTC	AGCTTATTCATCGCCACGTT
CTX-M gp 2	GGACGCTACCCCTGCTATT	CCAGCGTCAGATTTTTCAGG
CTX-M gp 8	TCGCGTTAAGCGGATGATGC	AACCCACGATGTGGGTAGC
CTX-M gp 9	CAAAGAGAGTGCAACGGATG	ATTGGAAAGCGTTCATCACC
CTX-M gp 25	GCAGGATGACATTCGGG	AACCCACGATGTGGGTAGC
TEM	ATTCTTGAAGACGAAAGGGCCTC	TTGGTCTGACAGTTACCAATGC
SHV	ATGCGTTATATTCGCCTGTG	GTTAGCGTTGCCAGTGCTCG
ACC	AACAGCCTCAGCAGCCGCTAA	TTCGCCGCAATCATCCCTAGC
MOX	GCTGCTCAAGGAGCACAGGAT	CACATTGACATAGGTGTGGTGC
EBC	TCGGTAAAGCCGATGTTGCGG	CTTCCACTGCGGCTGCCAGTT
FOX	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG
CIT	TGGCCAGAACTGACAGGCAAA	TTTCTCCTGAACGTGGCTGGC
DHA	CCGTACGCATACTGGCTTTGC	AACTTTCACAGGTGTGCTGGGT

E. coli and *K. pneumoniae* isolates exhibiting *ampC* phenotypes were screened for *ampC* genes (*bla$_{ACC}$, bla$_{MOX}$, bla$_{EBC}$, bla$_{FOX}$, bla$_{CIT}$, and bla$_{DHA}$*) by multiplex PCR assay and specific primers (See Table 2).

2.3.2 Imaging of Genotypic Analysis

PCR products were visualised on a 1% agarose gel containing ethidium bromide and UVP GelDoc-It^{TM} Imaging System.

2.3.3 Further Analysis of Genotypic Data

Isolates with negative bla$_{CTX-M}$ results but positive for bla$_{TEM}$ or bla$_{SHV}$ were sequenced to determine their ESBL status.

3 Results

3.1 Laboratory Participation

From September 2007, all laboratories across Wales were requested to collect and submit 1/6 of the *E. coli* or other coliforms resistant to 3rd generation cephalosporins isolated from community or hospital samples. In September 2008 characterisation was complete therefore no strains received after this date were included in the analysis.

Table 3 and Figure 1 show the numbers of isolates requested and sent/used from all laboratories in Wales

Figure 1: Percentage of requested isolates received/used from all laboratories

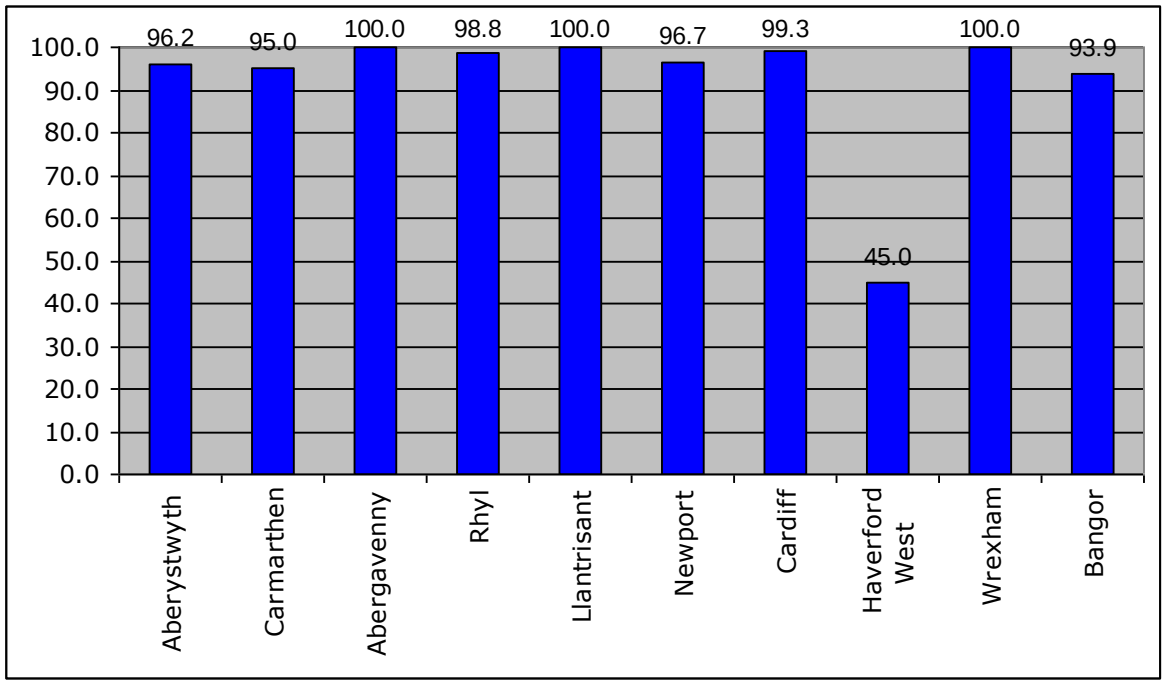


Table 3: Laboratory participation in targeted surveillance

Laboratory Name	No. of H-EC Requested	Sent/ Used	No. of H-COL Requested	Sent/ Used	No. of GP-EC Requested	Sent/ Used	No. of GP-COL Requested	Sent/ Used	Total Requested	Total Received/ Used
Aberystwyth	5	5/5	3	3/2	14	14/14	4	4/4	26	26/**25**
Bangor	17	17/15	15	15/13	34	31/31	16	19/19	82	82/**78**
Bridgend	13	1	11	1	19	4	8	0	51	6*
Cardiff	41	41/41	24	24/23	62	62/62	20	20/20	147	147/**146**
Carmarthen	20	8/8	15	14/8	30	17/17	11	5/5	76	40/**38**
Abergavenny	3	3/3	1	1/1	8	8/8	3	3/3	15	15/**15**
Rhyl	15	15/15	17	17/17	30	30/30	19	19/18	81	81/**80**
Llantrisant	12	12/12	8	8/8	17	17/17	4	4/4	41	41/**41**
Newport	21	21/21	46/44	46	24	24/24	29	29/27	120	120/**116**
Swansea	6	6	70	1	1	2	68	0	145	9*
Haverfordwest	2	2/2	11	10/0	3	3/3	20	5/4	36	20/**9**
Wrexham	21	21/21	7	7/7	32	32/32	7	2/2	67	62/**62**
TOTAL	176	151	228	137	274	247	209	105	887	731/**610**

* Too few isolates received for comparison.

Isolates excluded from the study if Proteus, Stenotrophomonas or Acinetobacter species. Also if *E. coli* sent as coliforms.

3.2 Cephalosporin Susceptibility

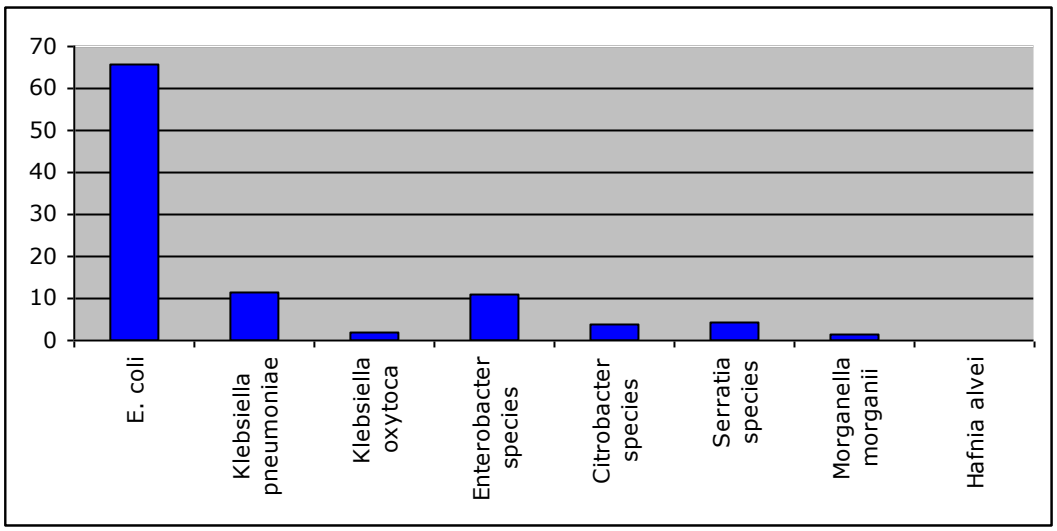
On confirmatory testing of the 610 isolates, 580 (95.1%) strains were resistant by MIC determination to either cefotaxime or ceftazidime. 30 (4.9%) were 3rd generation cephalosporin sensitive, with MICs to cefotaxime and ceftazidime of ≤1mg/L. The sensitive isolates originated in the following laboratories:

No. of Isolates	Laboratory	Percentage of strains sent
2	Abergavenny	13.3
5	Newport	4.3
16	Cardiff	10.9
3	Rhyl	3.8
2	Aberystwyth	8
1	Carmarthen	2.6
1	Bangor	1.3

3.3 Diversity of Species

Of the 580 3rd generation cephalosporin resistant isolates 381 (65.7%) were *Escherichia coli*, 78 (13.5%) were *Klebsiella* spp., 63 (10.8%) were *Enterobacter* spp., 22 (3.8%) were *Citrobacter* spp., 26 (4.5%) were *Serratia* spp., 9 (1.5%) were *Morganella* spp. and 1 (0.2%) were *Halfnia* spp. (Figure 2).

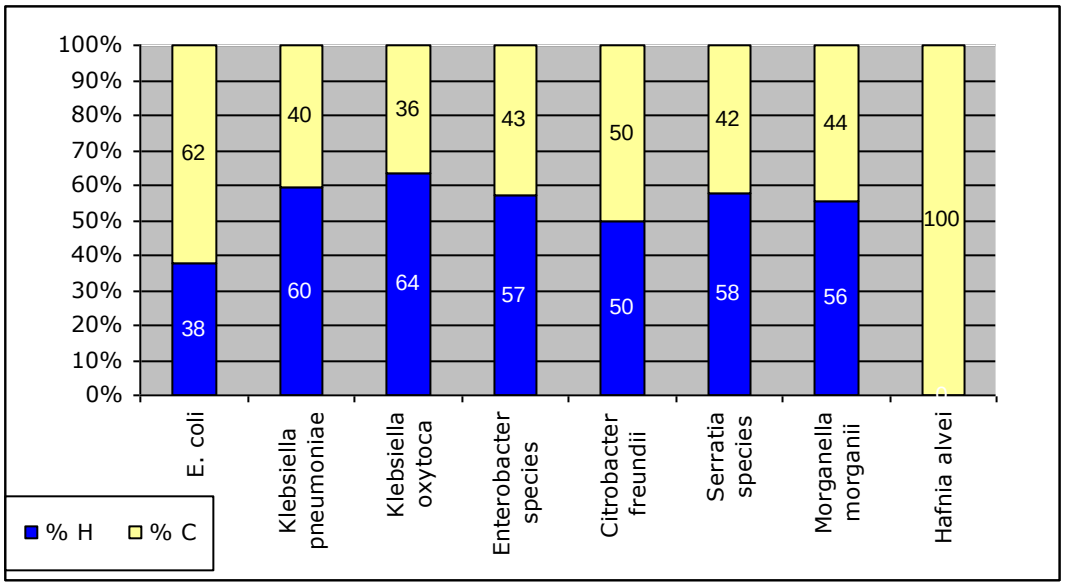
Figure 2: Diversity of coliform species received



Of the 580 3rd generation cephalosporin resistant isolates 322 (55.5%) were from the community (C) and 258 (44.5) were from hospitals (H). The percentage of hospital and community isolates was 37.8% and 62.2% in *E. coli*, 59.7% and 40.3% in *Klebsiella pneumoniae*, 63.6% and 36.4%

in *Klebsiella oxytoca*, 57.1% and 42.9% in *Enterobacter* spp., 50% and 50% in *Citrobacter* spp., 57.7% and 42.3% in *Serratia* spp., 55.6% and 44.4% in *Morganella* spp., and 0% and 100% in *Halfnia* spp. respectively (Figure 3).

Figure 3: Percentage of hospital (H) and community (C) isolates within species

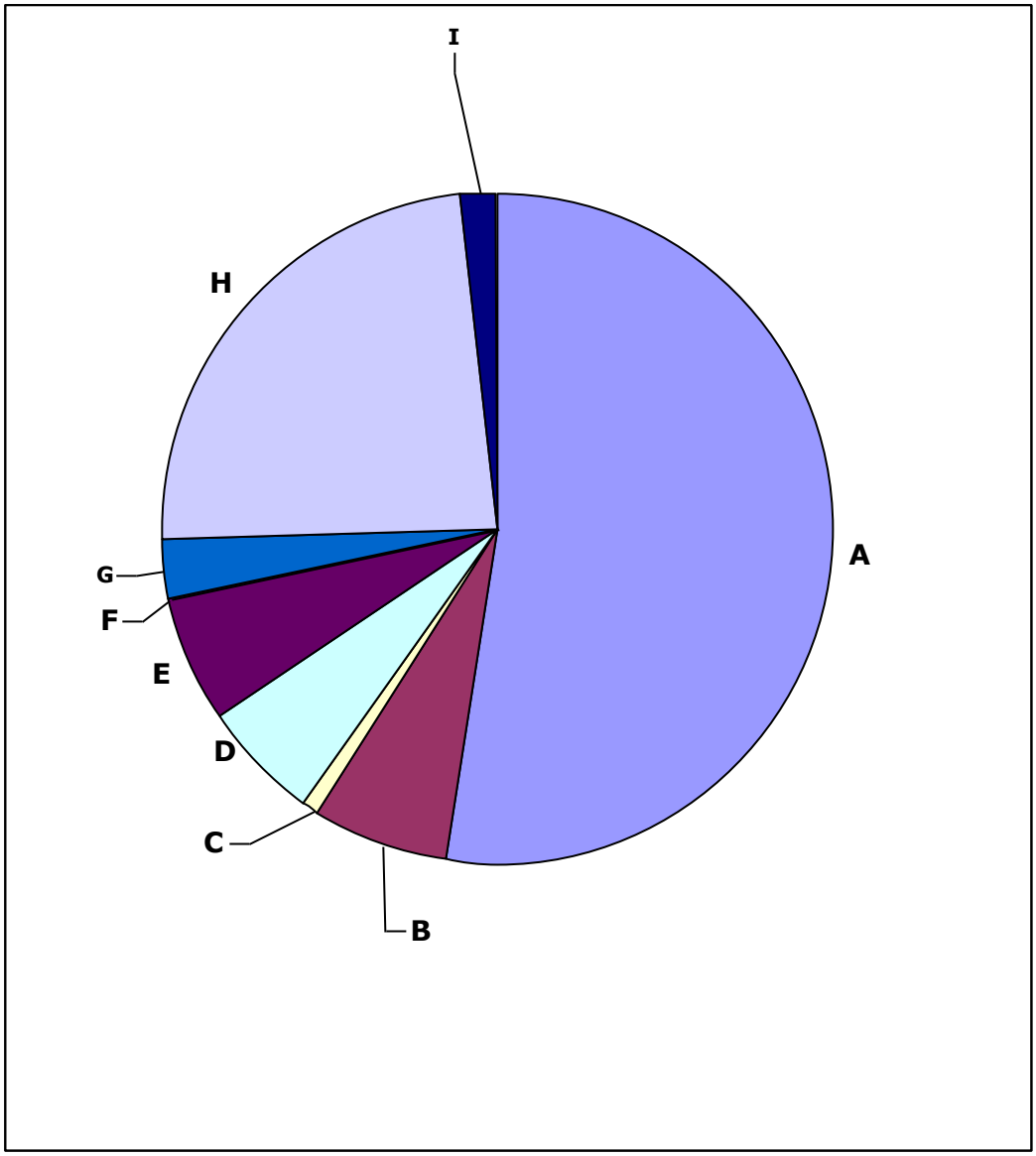


3.4 Resistance Mechanisms

3.4.1 Resistance Mechanisms found Wales-wide

Of the 580 3rd generation cephalosporin resistant isolates 346 (59.6%) contained CTX-M ESBLs, including 37 (6.4%) harbouring combined CTX-M plus hyper-production of chromosomal AmpC β-lactamases (c-ampC) and 4 (0.7%) harbouring CTX-M plus plasmid mediated AmpC β-lactamases (p-ampC) (Figure 4).

Figure 4: Breakdown of resistance mechanisms in All-Wales isolates

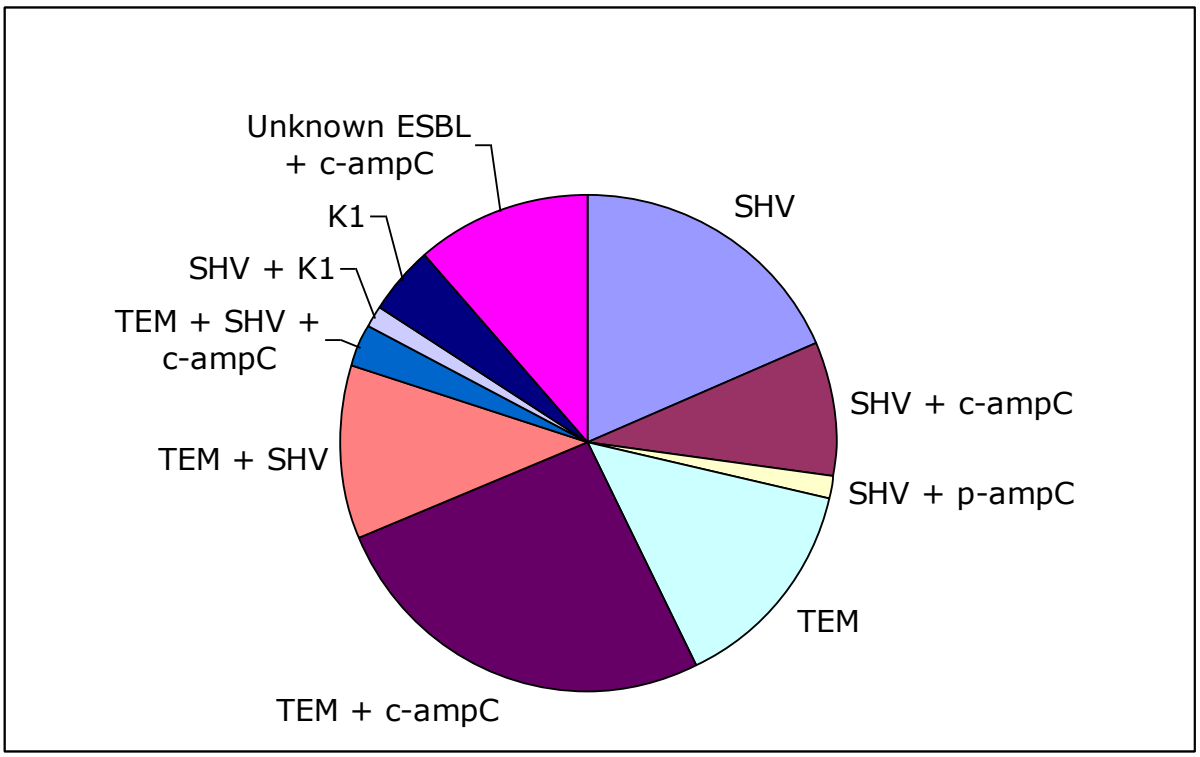


A	**CTX-M**
B	**CTX-M + c-ampC**
C	**CTX-M + p-ampC**
D	**Non-CTX-M ESBL**
E	**Other ESBL ± c-ampC**
F	**Other ESBL + p-ampC**
G	**p-ampC (Plasmidic)**
H	**c-ampC (hyper-produced chromosomal)**
I	**Unknown**

70 (12.1%) isolates contained ESBLs other than CTX-M, predominantly TEM and SHV (Figure 5), including 34 (5.8%) in combination with hyper expressed chromosomal ampC β-lactamases and 2 (0.3%) in combination with plasmid-mediated ampC β-lactamases. 225 (38.8%) isolates exhibited ampC β-lactamases either alone or in combination with another

mechanism. Of these, 16 (2.7%) isolates contained plasmid mediated ampC β-lactamases alone and 138 (23.8%) exhibited hyper production of chromosomal ampC β-lactamases alone. A further 71 (12.2%) isolates had a combination of chromosomal ampC β-lactamases with another mechanism. 5 (0.9%) isolates had plasmid-mediated ampC β-lactamases with another mechanism. 10 (1.7%) of isolates contained an unknown mechanism of resistance.

Figure 5: Breakdown of resistance mechanisms other than CTX-M



These figures are in line with a similar UK-based study which report 44.7% of 3rd generation cephalosporin resistant *Enterobacteriaceae* containing CTX-M ESBLs, 13.3% with non-CTX-M ESBLs and 16.9% with high-level ampC β-lactamases and 25.2% with mechanisms other than ESBL or ampC enzymes (Potz et al 2006 JAC 58:320-326).

3.4.2 Single and Multiple Resistance Mechanisms

In 495 (85.3%) of the 580 isolates 3rd generation cephalosporin resistance was conferred by a single resistance mechanism compared to 85 (14.7%) where multiple mechanisms were found (Table 4).

Table 4: Breakdown of resistance mechanisms in 580 3rd generation cephalosporin resistant *Enterobacteriaceae*

Resistance Mechanism	No	%
CTX-M	305	52.6
CTX-M + c-ampC	37	6.4
CTX-M + p-ampC	4	0.7
SHV	13	2.2
SHV + c-ampC	6	1
SHV + p-ampC	1	0.2
TEM	10	1.7
TEM + c-ampC	18	3.1
TEM + SHV	8	1.4
TEM + SHV + c-ampC	2	0.3
p-ampC	16	2.8
c-ampC	138	23.8
SHV + K1	1	0.2
K1	3	0.5
Unknown resistance mechanism	10	1.7
Unknown ESBL + c-ampC	8	1.4
TOTAL	580	100.00

3.4.3 Resistance Mechanisms Prevalent per Laboratory

The following graph shows the prevalence of isolates with each resistance mechanism per laboratory (Figure 6). These are percentages of 3rd generation cephalosporin resistant isolates received from each laboratory. The all-Wales figure is added as a guide to national prevalence.

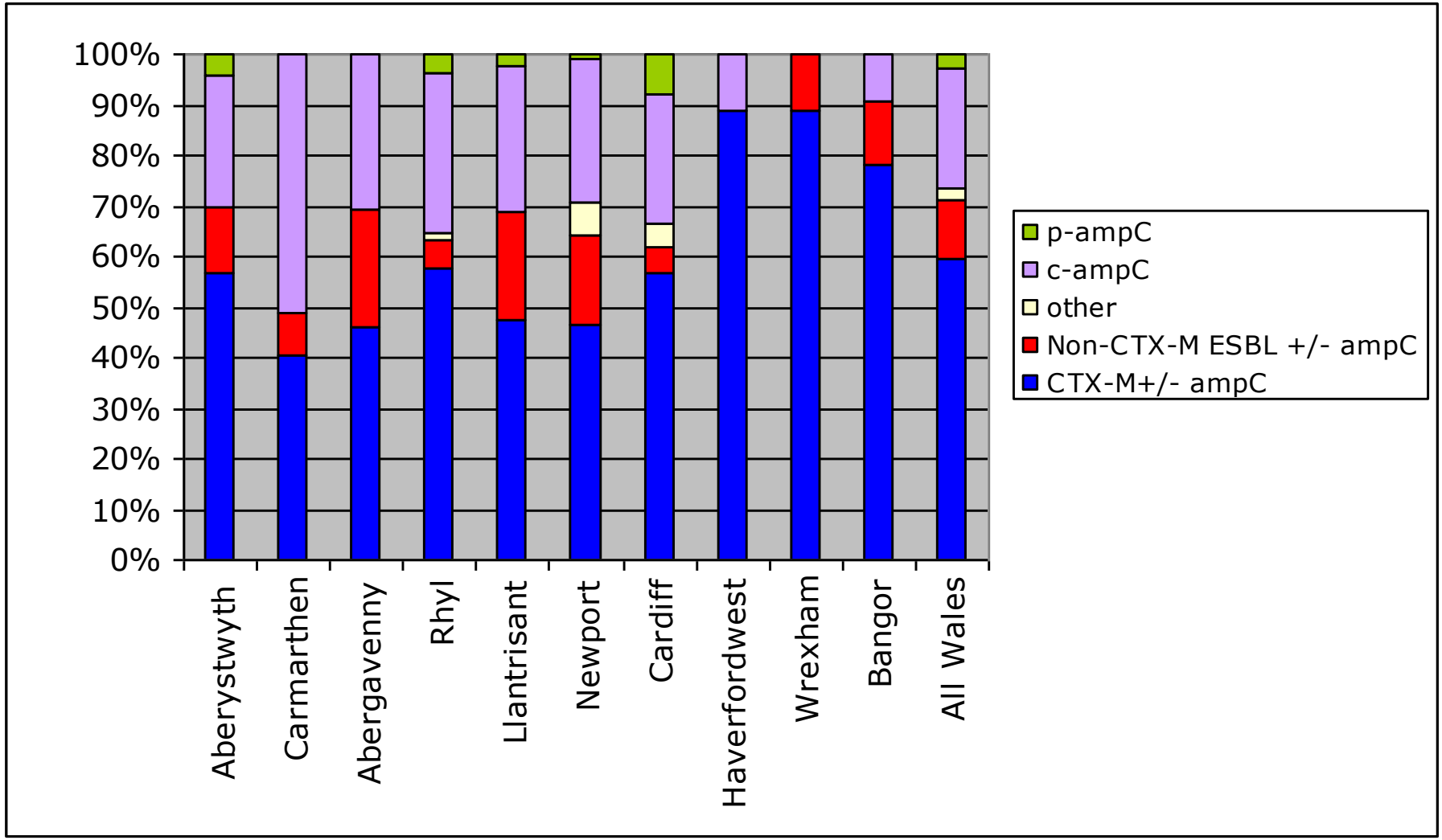
In Haverfordwest and Wrexham 88.9% and 88.7% of 3rd generation cephalosporin resistant isolates contained CTX-M genes, approximately double (44.7%) that found in London in a similar study (Potz *et al.*). In Haverfordwest CTX-M was the only ESBL found, whilst in Wrexham no isolate hyper-expressed chromosomal ampC. Prevalence of CTX-M genes in isolates from Newport, Llantrisant and Carmarthen were the lowest in Wales and similar to UK figures.

The percentage of 3rd generation cephalosporin resistant isolates harbouring ESBLs in each laboratory is shown below, with the highest percentage in Wrexham (100%) and the lowest in Carmarthen (48.6%).

Table 5: 3rd generation cephalosporin resistant *Enterobacteriaceae* exhibiting ESBL phenotype and genotype

Laboratory	No ESBL	% ESBL
All Wales	346	59.7
Aberystwyth	16	69.6
Carmarthen	18	48.6
Abergavenny	9	69.2
Rhyl	48	63.1
Llantrisant	29	69
Newport	72	64.3
Cardiff	80	62
Haverfordwest	8	88.8
Wrexham	62	100
Bangor	70	90.1

Figure 6: Breakdown of resistance mechanisms per laboratory



CTX-M type ß-lactamases

Of the isolates containing CTX-M genes in Llantrisant approximately half were in combination with hyper-expressed ampC ß-lactamases (Figures 7 & 8, Tables 6 & 7). In Haverfordwest, approximately one third of the isolates containing CTX-M genes also hyper-expressed their native ampC ß-lactamases. In isolates containing CTX-M genes from all other laboratories the prevalence in combination with hyper-expressed ampC ß-lactamases was less than 10%. Isolates with CTX-M genes in combination with plasmid-mediated ampC ß-lactamases were found at Cardiff, Newport and Bangor.

Figure 7: Percentage of isolates with CTX-M ESBL (total) per laboratory

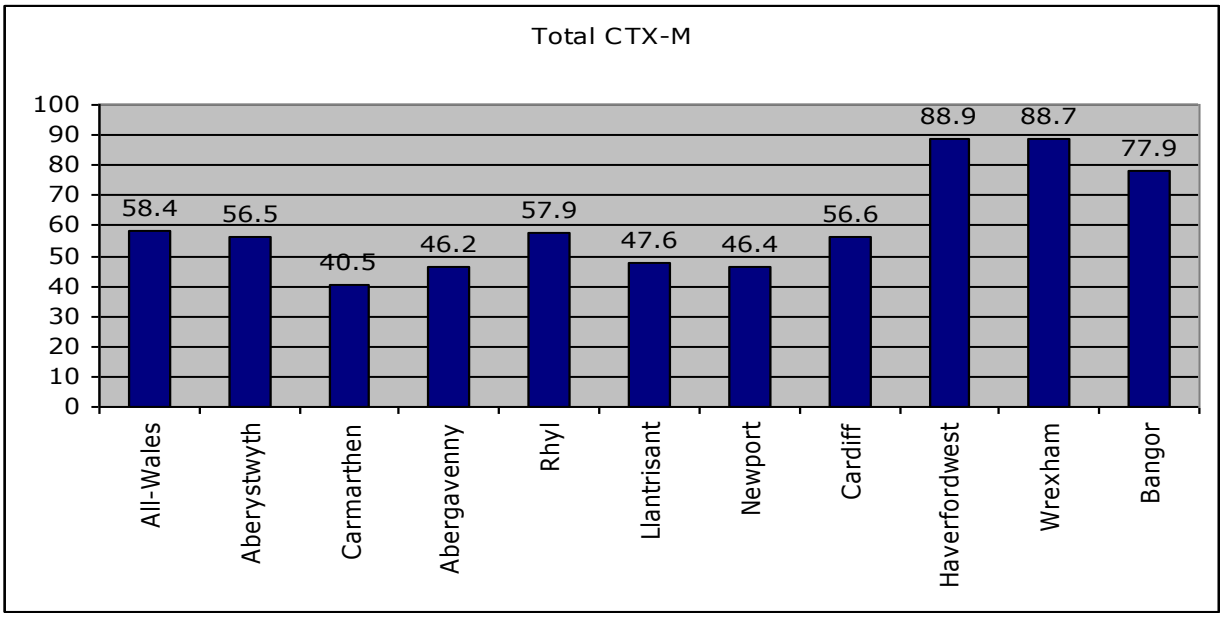


Table 6: Total numbers of isolates with CTX-M ESBL per laboratory

Laboratory	CTX-M
All Wales	346
Aberystwyth	13
Carmarthen	15
Abergavenny	6
Rhyl	44
Llantrisant	20
Newport	52
Cardiff	73
Haverfordwest	8
Wrexham	55
Bangor	60

Figure 8: Breakdown of isolates with CTX-M ESBL in combination with either hyper-expressed chromosomal ampC or plasmid-mediated ampC ß-lactamases per laboratory

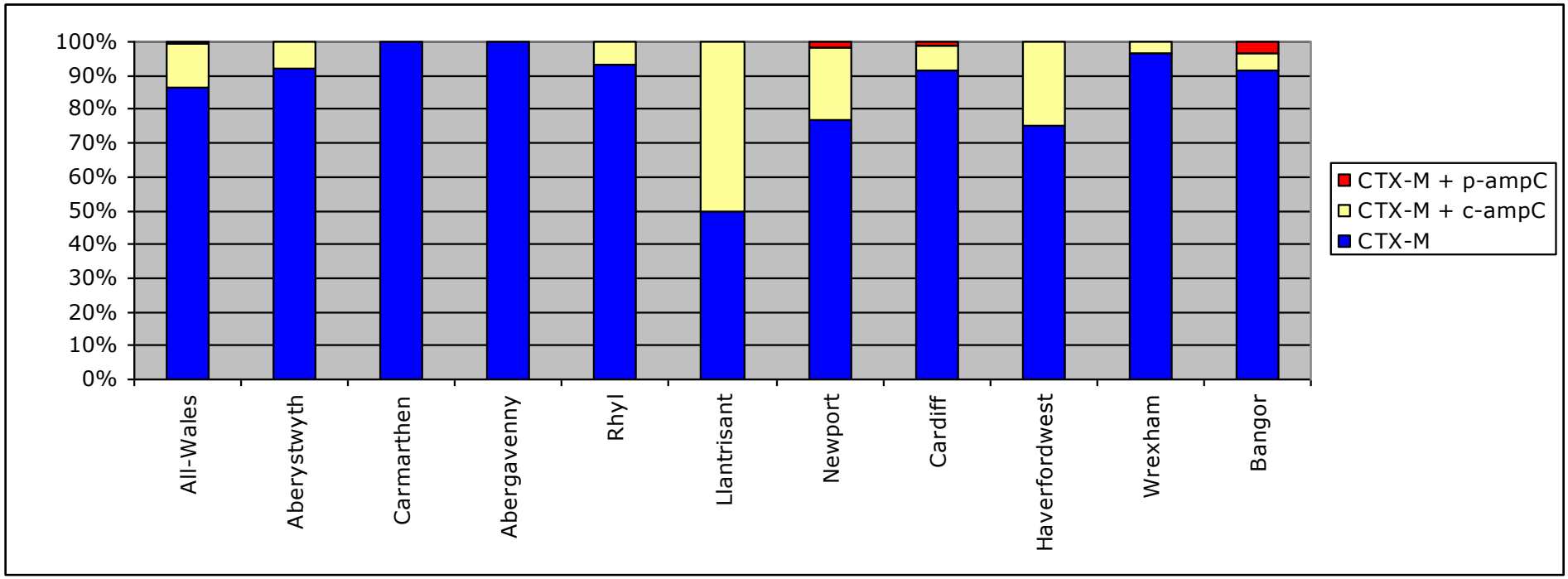


Table 7: Numbers of isolates with CTX-M ESBL in combination with either hyper-expressed chromosomal ampC or plasmid-mediated ampC ß-lactamases per laboratory

Laboratory	CTX-M alone	CTX-M + c-ampC	CTX-M + p-ampC
All Wales	250	34	2
Aberystwyth	12	1	0
Carmarthen	15	0	0
Abergavenny	6	0	0
Rhyl	41	3	0
Llantrisant	10	10	0
Newport	40	11	1
Cardiff	67	5	1
Haverfordwest	6	2	0
Wrexham	53	2	0
Bangor	55	3	2

K1 ß-lactamases

K. oxytoca isolates exhibiting K1 ß-lactamase were found in Cardiff and Newport laboratories only (Figure 9, Table 8).

Figure 9: The percentage of isolates with K1 ß-lactamases per laboratory

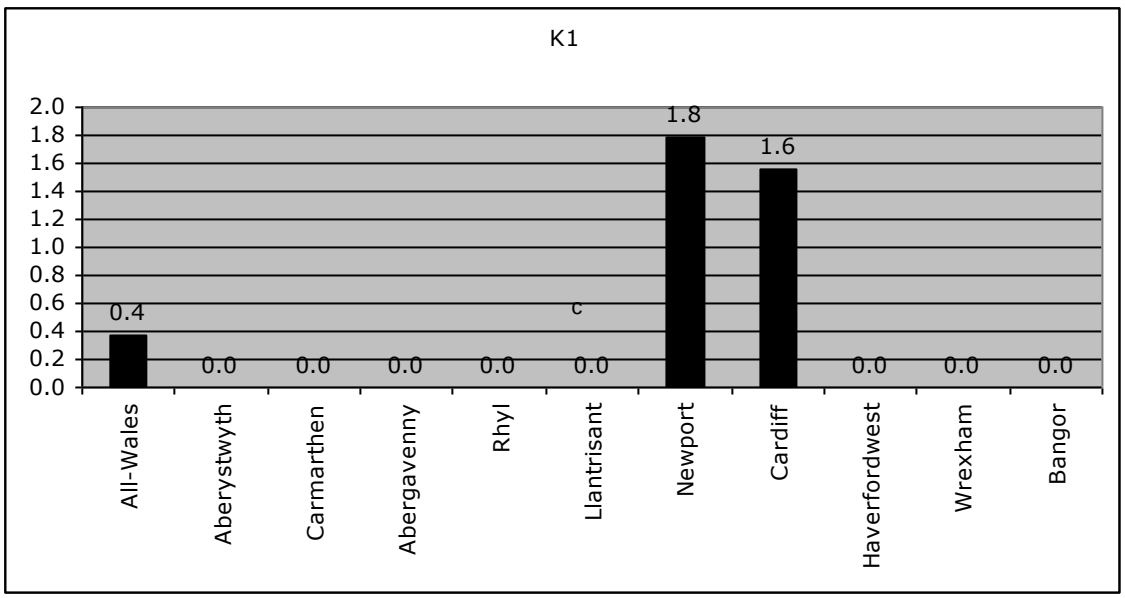


Table 8: Total numbers of isolates with K1 ß-lactamases per laboratory

Laboratory	K1
All Wales	0
Aberystwyth	0
Carmarthen	0
Abergavenny	0
Rhyl	0
Llantrisant	0
Newport	2
Cardiff	2
Haverfordwest	0
Wrexham	0

AmpC ß-lactamases

Hyper- expressed chromosomal ampC ß-lactamases

In Carmarthen and Llantrisant over half of 3rd generation cephalosporin resistant isolates had hyper expressed ampC ß-lactamases (Figures 10 -11, Table 9 & 10). Wrexham had the fewest isolates containing hyper expressed ampC ß-lactamases.
In Wrexham all strains containing hyper expressed chromosomal AmpC were in combination with another resistance mechanism. In Haverfordwest around two thirds of isolates hyper expressing ampC ß-lactamases also had a further resistance mechanism present. In Llantrisant and Newport just over half of the isolates containing hyper expressed ampC ß-lactamases were in combination with another resistance mechanism. In all other laboratories the majority of isolates with hyper expressed ampC ß-lactamases had no other resistance mechanism present.

Plasmid-mediated ampC ß-lactamases

Isolates containing plasmid-mediated ampC ß-lactamases were found in Aberystwyth, Rhyl, Llantrisant, Newport, Bangor and Cardiff laboratories but not in Carmarthen, Abergavenny, Haverfordwest or Wrexham laboratories. In most cases the plasmid-mediated ampC ß-lactamase was present alone (Figures 10 -11, Table 8 & 9).

Figure 10: The percentage prevalence of isolates with ampC ß-lactamases per laboratory

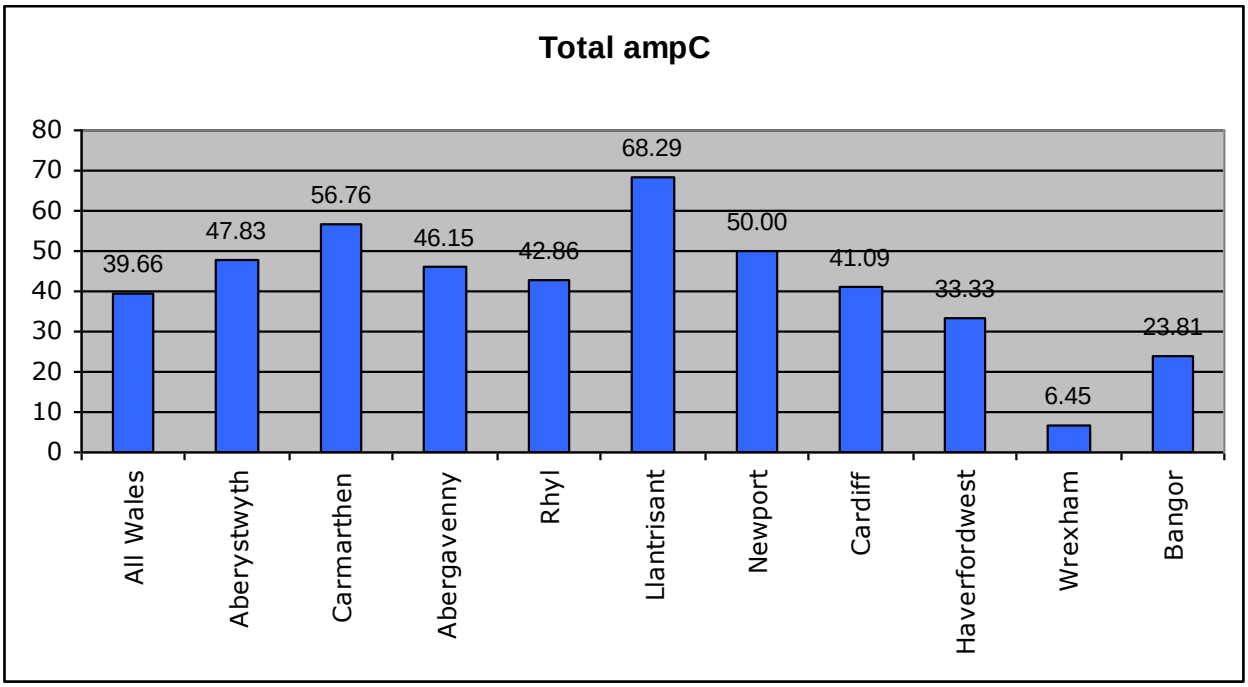
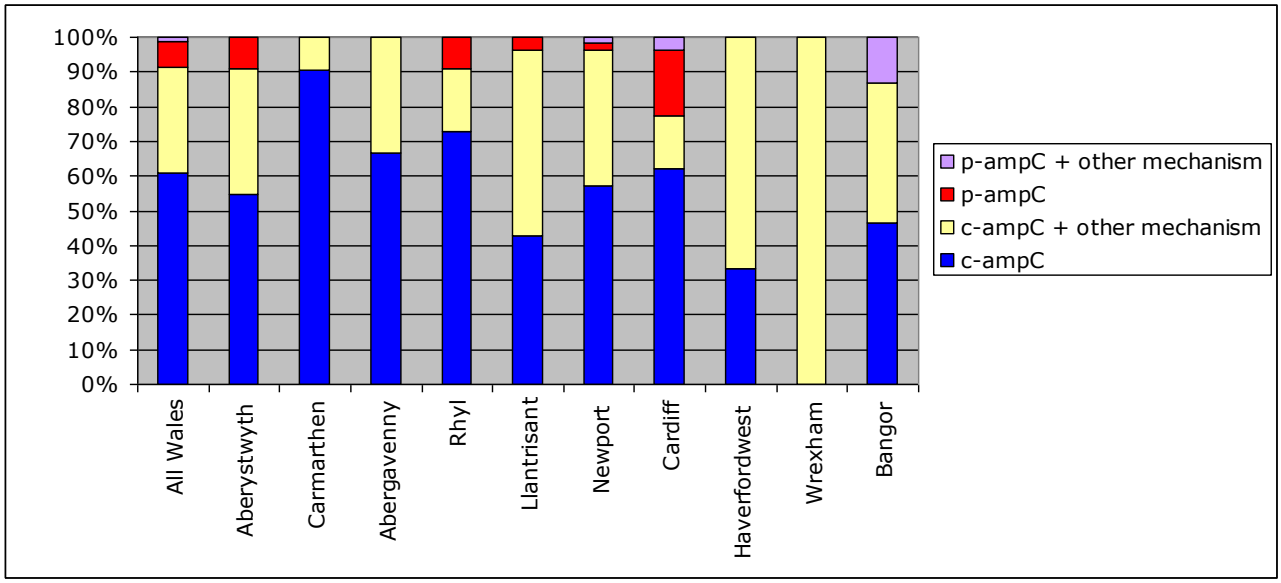


Table 9: Total number of isolates with ampC ß-lactamases (chromosomal & plasmid mediated) per laboratory

Laboratory	ampC
All Wales	230
Aberystwyth	11
Carmarthen	21
Abergavenny	6
Rhyl	33
Llantrisant	28
Newport	56
Cardiff	53
Haverfordwest	3
Wrexham	4
Bangor	15

Figure 11: Breakdown of isolates with ampC ß-lactamases alone or in combination with other resistance mechanisms per laboratory



c-ampC = hyper expressed chromosomal ampC
p-ampC = plasmid mediated ampC

Table 10: Numbers of isolates with ampC ß-lactamases either alone or in combination with other resistance mechanisms per laboratory

Laboratory	c-ampC alone	c-ampC + other resistance mechanism	p-ampC alone	p-ampC + other resistance mechanism
All Wales	138	71	16	5
Aberystwyth	6	4	1	0
Carmarthen	19	2	0	0
Abergavenny	4	2	0	0
Rhyl	24	6	3	0
Llantrisant	12	15	1	0
Newport	32	22	1	1
Cardiff	33	8	10	2
Haverfordwest	1	2	0	0
Wrexham	0	4	0	0
Bangor	7	6	0	2

c-ampC = hyper expressed chromosomal ampC
p-ampC = plasmid mediated ampC

SHV & TEM type ESBL ß-lactamases

Isolates from most laboratories (except Haverfordwest & Aberystwyth) contained SHV ESBL genes, with most likely to contain other resistance mechanisms, especially ampC ß-lactamases. There was one isolate containing a SHV ESBL and plasmid-mediated ampC ß-lactamase, originating from Cardiff (Figures 12 – 14, Table 11).

All laboratories, except Haverfordwest, had isolates which contained TEM ESBLs, the majority of which were in combination with other resistance mechanisms. Two isolates from Llantrisant laboratory contained both TEM and SHV ESBL genes plus hyper-expressed ß-lactamases (Figures 12 – 14, Table 10).

Figure 12: The percentage prevalence of isolates with SHV ESBL per laboratory

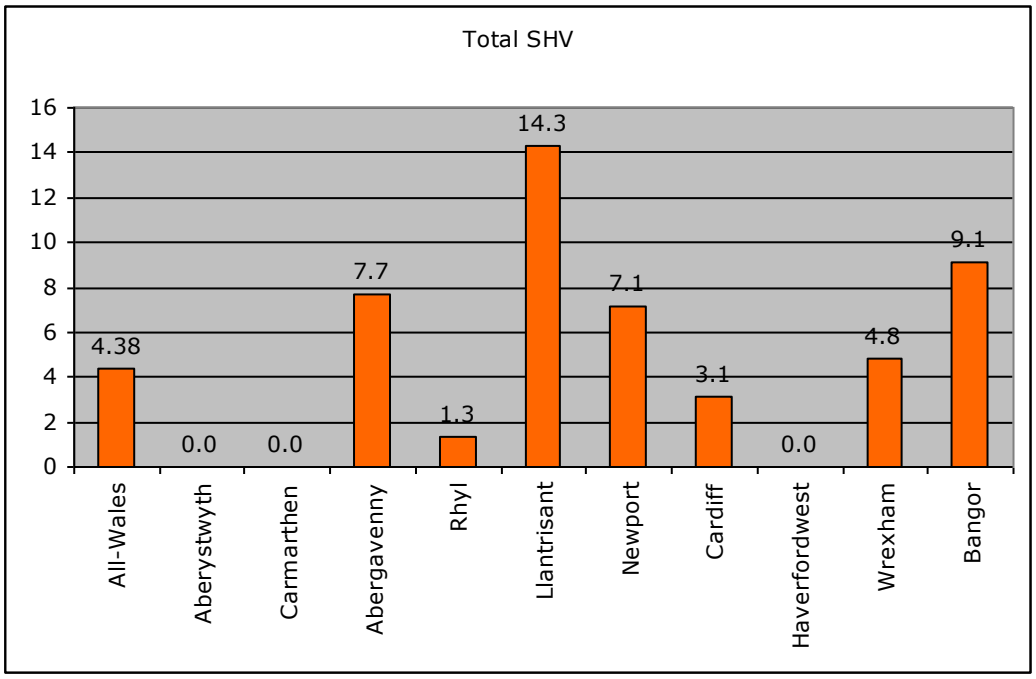


Figure 13: The percentage prevalence of isolates with TEM ESBL per laboratory

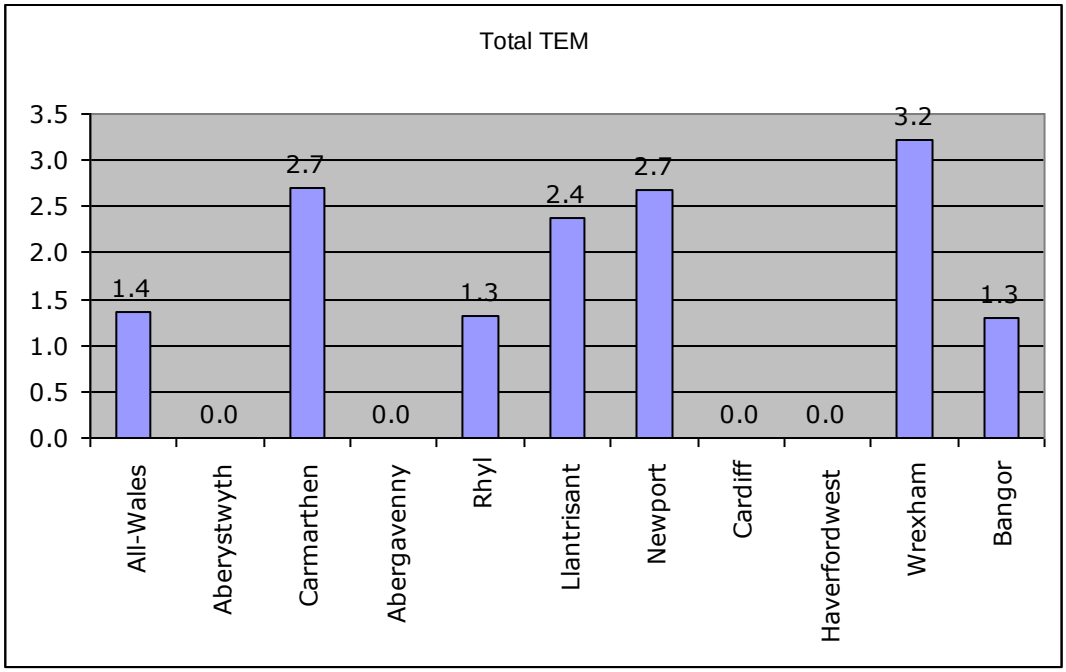
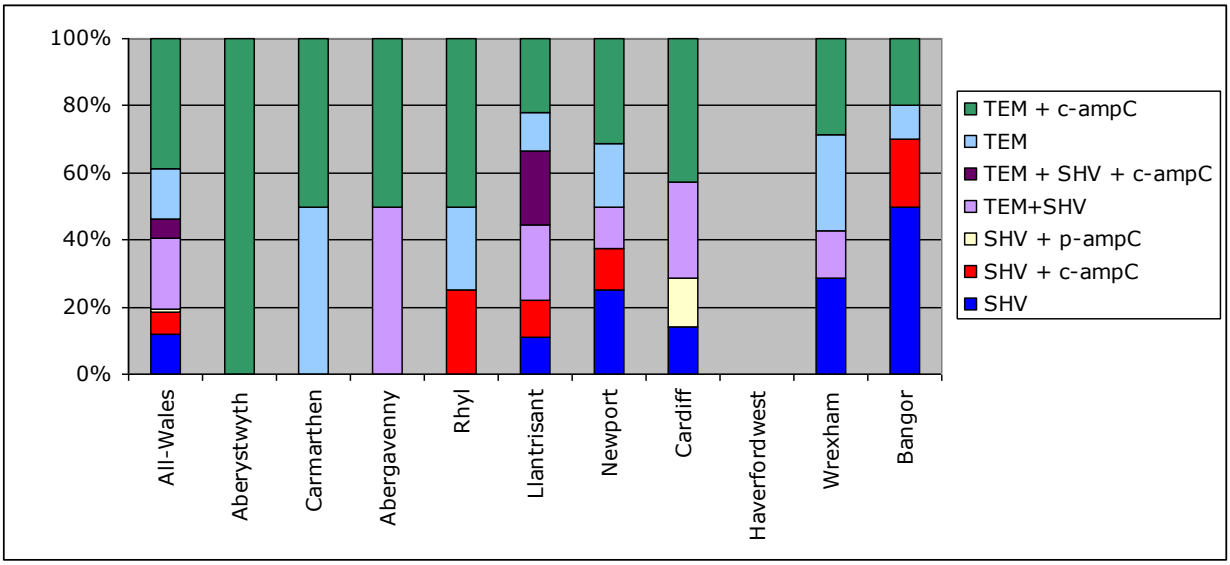


Table 11: Numbers of isolates with TEM and/or SHV ESBL either alone or in combination with ampC ß-lactamases per laboratory

Laboratory	SHV alone	SHV + c-ampC	SHV + p-ampC	TEM + SHV	TEM + SHV + c-ampC	TEM alone	TEM + c-ampC
All Wales	8	4	1	8	2	8	17
Aberystwyth	0	0	0	0	0	0	1
Carmarthen	0	0	0	0	0	1	1
Abergavenny	0	0	0	1	0	0	1
Rhyl	0	1	0	0	0	1	2
Llantrisant	1	1	0	2	2	1	2
Newport	4	2	0	2	0	3	5
Cardiff	1	0	1	2	0	0	3
Haverfordwest	0	0	0	0	0	0	0
Wrexham	2	0	0	1	0	2	2
Bangor	5	2	0	0	0	1	2

c-ampC = hyper expressed chromosomal ampC
p-ampC = plasmid mediated ampC

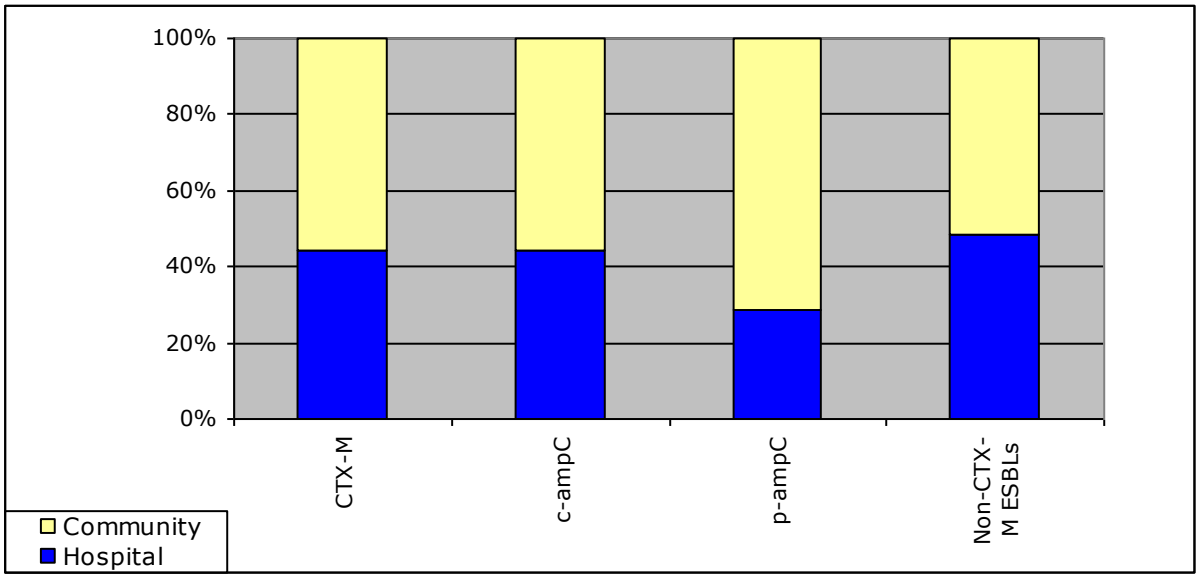
Figure 14: Breakdown of isolates with TEM & SHV ESBL plus ampC ß-lactamases per laboratory



3.4.4 Resistance Mechanisms Predominant in Hospital and Community

Of the isolates with ESBLs (CTX-M, TEM, SHV) genes detected, either alone or in combination with ampC β-lactamases, approximately half were isolated from hospital and community strains. However in the case of plasmid-mediated ampC β-lactamases, 15 (71.4%) were isolated from Community strains compared with 6 (28.6%) from Hospital strains (Figure 15). This suggests that plasmid-mediated ampC β-lactamases are more prevalent in the community.

Figure 15: Resistance mechanisms prevalent in hospital and community isolates



3.4.5 Species Specific Resistance Mechanisms

CTX-M genes were found predominantly in *E. coli* and *Klebsiella* spp. (Table 12, Figure 16). CTX-M enzymes account for 74% of the cephalosporin resistance in *E. coli* and 59% in *Klebsiella* spp.. They were less common in *Enterobacter* spp. (18.8%), *Citrobacter* spp. (31.8%), *Serratia* spp. (3.4%) and *Morganella* spp. (11.1%).

3rd generation cephalosporin resistance in *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and *Morganella* spp. was mainly conferred by hyper production of chromosomal β-lactamases. These enzymes were sometimes found to contribute to 3rd generation cephalosporin resistance in combination with other resistance mechanisms in 23.4% of *Enterobacter* spp., 27.3% of *Citrobacter* spp., 17.2% of *Serratia* spp. and 33.3% of *Morganella* spp..

Plasmid mediated ampC β-lactamases were found only in *E. coli* (4%) and *Klebsiella* spp. (7.7%), mainly alone but sometimes in combination with

CTX-M. It is understood that the plasmids known to carry CTX-M also harbour other resistance genes such as CIT (ampC β-lactamase). One plasmid mediated ampC β-lactamase is in combination with a SHV ESBL in an *E. coli* strain (Table 11).

Of 78 Klebsiella spp. 10 were K. oxytoca, 4 of which exhibited a phenotype indicating a hyper-produced KOXY (K1) β-lactamase.

Figure 16: Percentage of resistance mechanisms in specific species

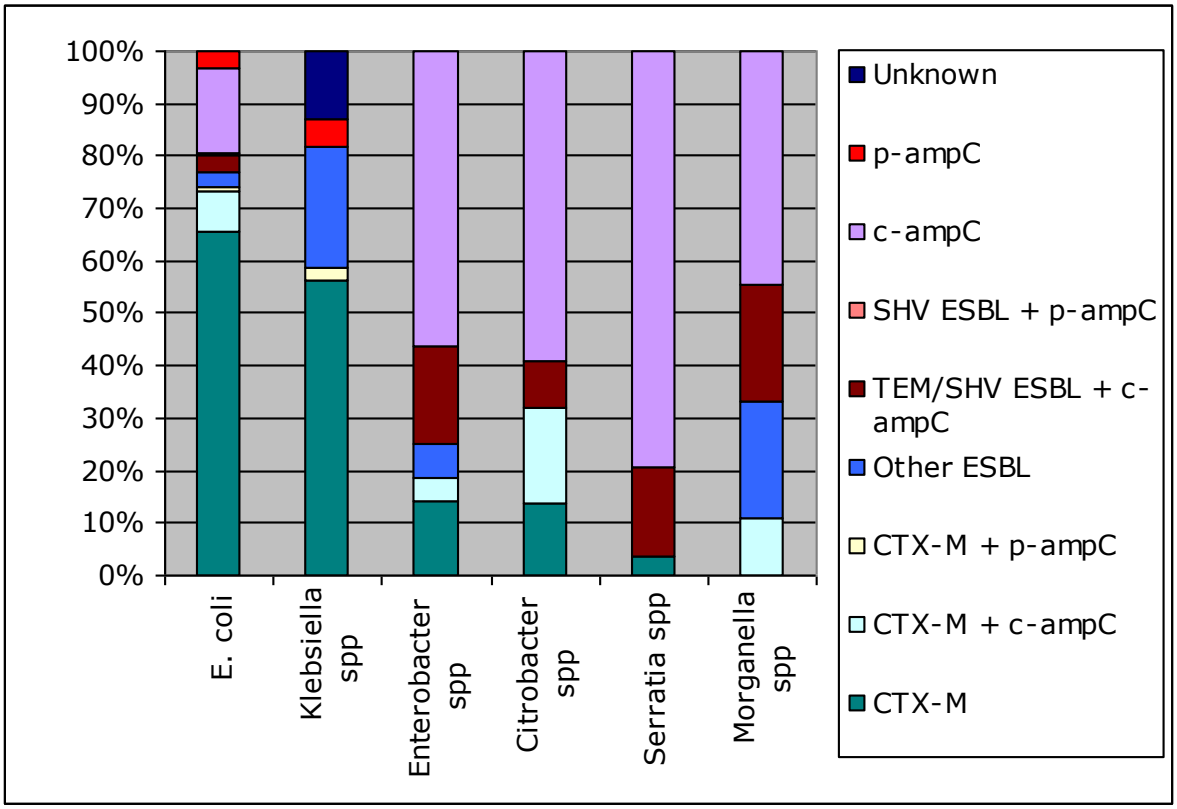


Table 12: Percentage of resistance mechanisms in specific species

Species	CTX-M	CTX-M + c-ampC	CTX-M + p-ampC	Other ESBL	TEM/SHV ESBL + c-ampC	SHV ESBL + p-ampC	c-ampC	p-ampC	Unknown
E. coli	65.8	7.7	0.5	2.9	3.2	0.3	16.4	3.2	0.0
Klebsiella spp.	56.4	0.0	2.6	23.1	0.0	0.0	0.0	5.1	13.2
Enterobacter spp.	14.1	4.7	0.0	6.3	18.8	0.0	56.3	0.0	0
Citrobacter spp.	13.6	18.2	0.0	0.0	9.1	0.0	59.1	0.0	0
Serratia spp.	3.4	0.0	0.0	0.0	17.2	0.0	79.3	0.0	0
Morganella spp.	0.0	11.1	0.0	22.2	22.2	0.0	44.4	0.0	0

3.4.6 Diversity of CTX-M type groups

CTX-M type ß-lactamases can be divided into 5 groups according to sequence similarity (Figure 17).

346 isolates in total contained CTX-M genes, comprising 313 (90.5%) CTX-M group 1, 5 (1.5%) CTX-M group 2, 3 (0.8%) CTX-M group 8 and 25 (7.2%) group 9 (Figure 18).

CTX-M-15 (CTX-M group 1 type) is the most prevalent type of CTX-M in the UK and Europe. The prevalence of CTX-M group 1 genes in CTX-M producing isolates from Welsh laboratories (90.5%) is similar to that found in UK data, where 86.4% were CTX-M group 1 genes.

CTX-M group 2 are highly prevalent in South America, Israel and Japan but are very rare in UK patients. In a UK study 0.5% of CTX-M producing isolates contained CTX-M group 2 genes. In Wales, the isolates containing CTX-M groups 2 genes originated from three laboratories so it may possible that they are separate introductions. However three were isolated from in-patients at Newport Laboratory, which may constitute patient to patient transmission (Figure 19).

CTX-M group 2 genes constitute 5.8% of isolates containing CTX-M genes in strains from Newport Laboratory, 1.8% of isolates from Wrexham and 1.4% from Cardiff (Figure 19, Table 6).

CTX-M-9 genes are the most prevalent CTX-M type in Spain and have been found in considerable numbers in most European countries including the UK, where the 2nd most prevalent type after CTX-M 15 is CTX-M-14, a member of group 9 (Figure 17). The prevalence of CTX-M group 9 CTX-M in Wales (7.2%) is similar but slightly lower to that in the UK (12.8%). Percentage of CTX-M group 9 genes ranged from 3.6% to 9.5% of CTX-M containing isolates. However, 3 isolates contained CTX-M group 9 genes from Carmarthen and 9 from Bangor, representing 20% and 15% of isolates containing CTX-M genes and 7.9%, 11.7% of 3GC resistant strains in the respective areas. (Figure 19, Table 13).

CTX-M group 8 genes are rare in the UK. CTX-M group 8 genes were found in 3 isolates from Bangor only. This represented 5% of the CTX-M gene containing isolates and 3.9% of the total 3GC resistant isolates from Bangor.

Figure 17: Dendogram of CTX-M groups

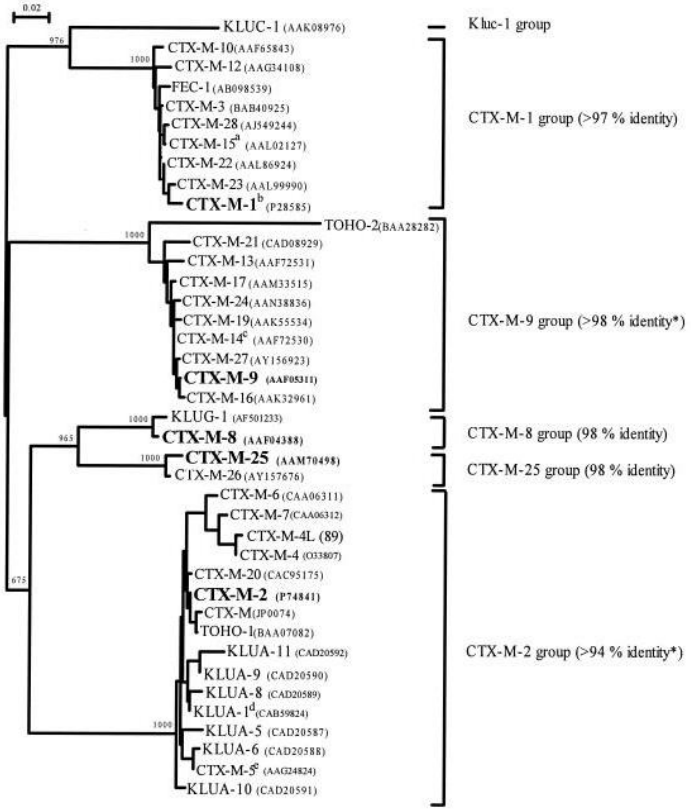


Figure 18: Prevalence of CTX-M gene groups

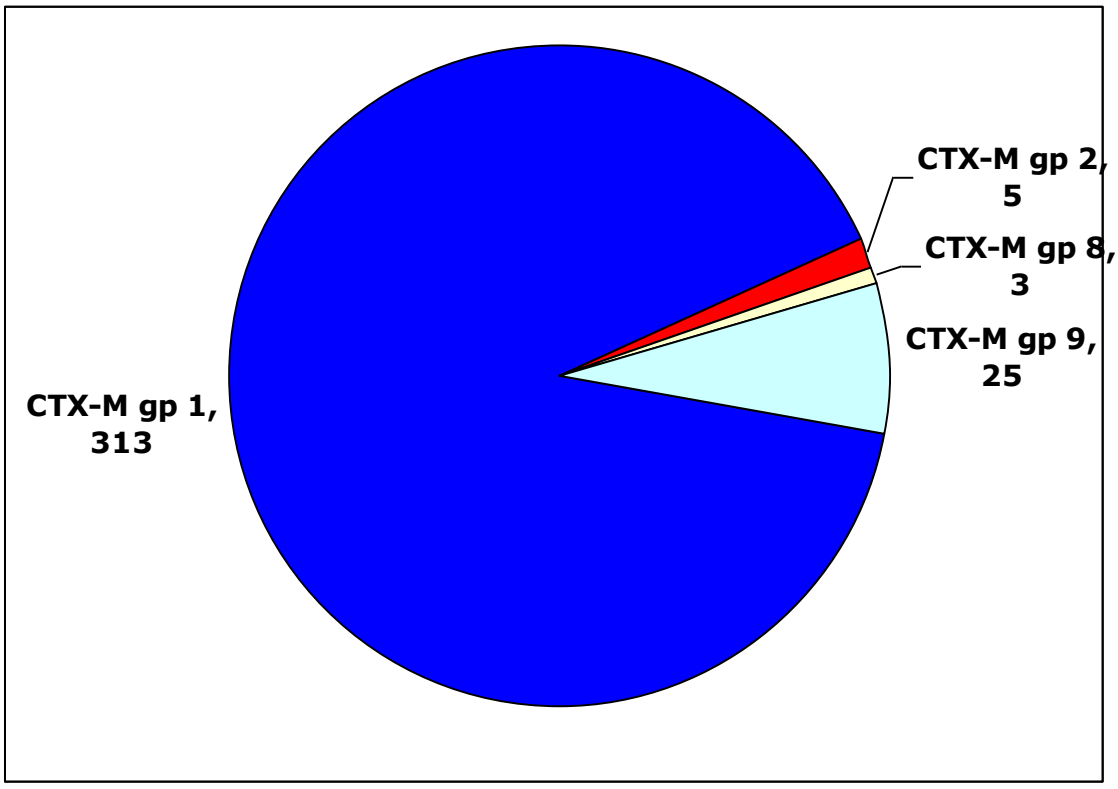


Figure 19: CTX-M gene groups per laboratory

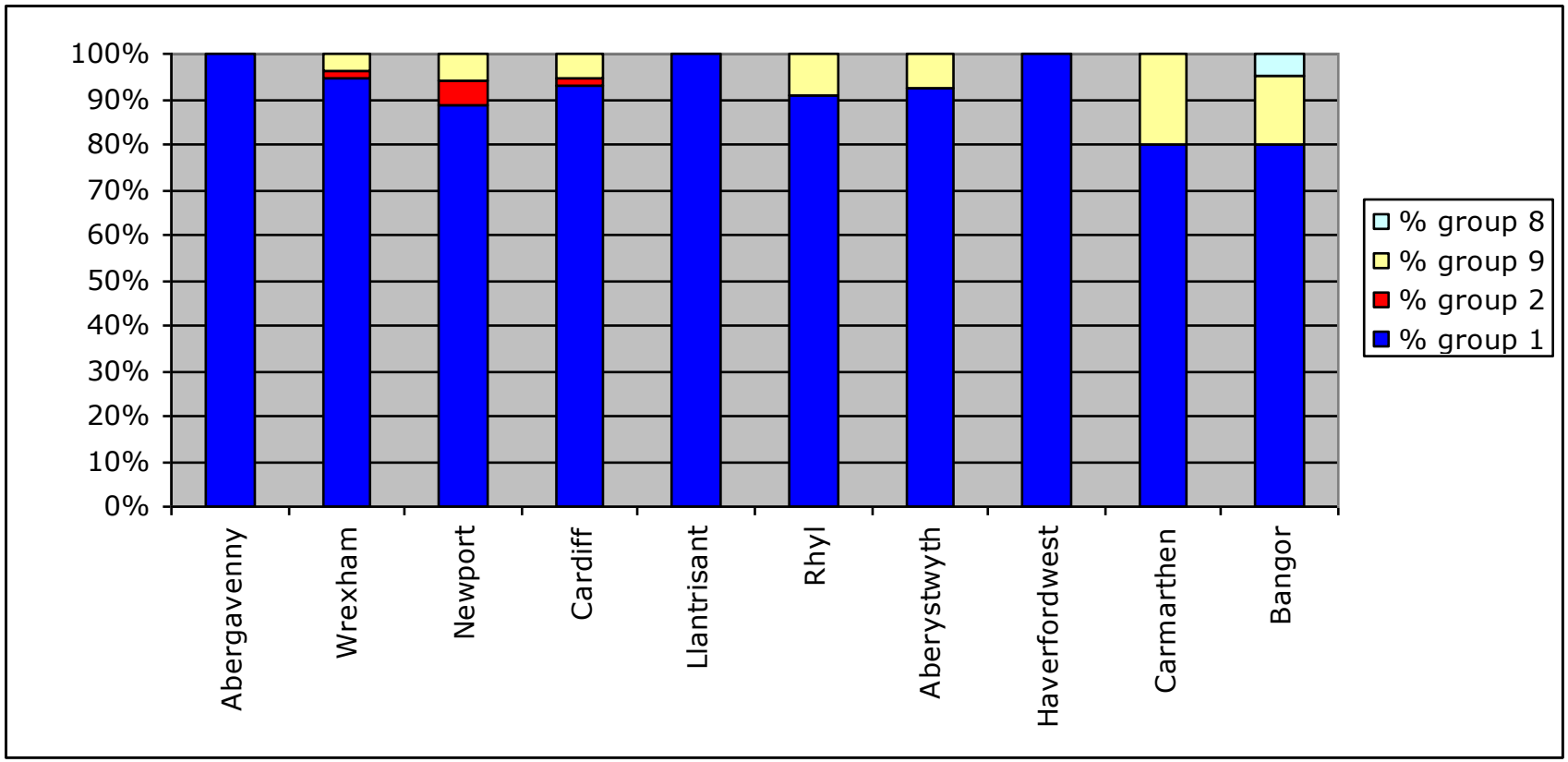


Table 13: CTX-M group diversity per laboratory

Laboratories	Total No. with CTX-M	No of Group 1	% Group 1	No of Group 2	% Group 2	No of Group 9	% Group 9	No of Group 8	% Group 8
Abergavenny	6	6	100	0	0	0	0	0	0
Wrexham	55	52	94.5	1	1.8	2	3.6	0	0
Newport	52	46	88.5	3	5.8	3	5.8	0	0
Cardiff	73	69	93.3	1	1.4	4	9.1	0	0
Llantrisant	20	20	100	0	0	0	9.5	0	0
Rhyl	44	40	90.9	0	0	4	9.1	0	0
Aberystwyth	13	12	92.3	0	0	1	7.7	0	0
Haverfordwest	8	8	100	0	0	0	0	0	0
Carmarthen	15	12	80	0	0	3	20	0	0
Bangor	60	48	80	0	0	9	15	3	5
All-Wales	346	312	90.2	5	1.4	26	7.5	3	0.9

3.4.7 Correlation of Cephalosporin MICs with Resistance Mechanisms

ESBL ß-lactamases

In isolates containing CTX-M (all groups), ceftazidime and cefotaxime geometric MIC means were 9.4 and 22 respectively. This data confirms that CTX-M enzymes hydrolyse cefotaxime more rapidly than ceftazidime (Table 14, Figure 20 - 23). Isolates containing other ESBL enzymes exhibit a higher GeoMean to ceftazidime than cefotaxime (Figure 24). In general isolates containing CTX-M genes, especially CTX-M group 1, are more resistant to cefotaxime than those containing other ESBLs.

Some isolates were found to be sensitive to either ceftazidime or cefotaxime. For isolates containing CTX-M group 1, group 2, group 8 and group 9 enzymes, 4.5%, 40%, 0% and 12% were sensitive to ceftazidime whilst 1.6%, 40%, 0% and 0% were sensitive to cefotaxime. In isolates harbouring other ESBL enzymes 7% and 21% were sensitive to ceftazidime and cefotaxime respectively.

Table 14: Ceftazidime (CAZ) MIC ranges and GeoMeans for various resistance mechanisms

Resistance mechanism	CAZ MIC Range (mg/L)	Geo Mean	CTX MIC Range (mg/L)	Geo Mean
CTX-M gp 1	0.5 - 256	20.7	0.5 - 256	61.4
CTX-M gp 2	1 – 16	3.5	0.12 - 64	3.5
CTX-M gp 8	2 - 64	12.7	8 - 32	20.2
CTX-M gp 9	1 - 256	8.5	4 – 256	55
Total CTX-M	0.5 - 256	9.4	0.5 – 256	22
Non-CTX-M ESBL	0.12 – 256	17.6	0.06 – 256	9.4
c-ampC	0.25 – 256	12.5	0.015 – 256	6.9
p-ampC	2 - 256	81	2 - 256	14.9

Figure 20: Ceftazidime and cefotaxime MIC range in isolates containing CTX-M group 1 genes

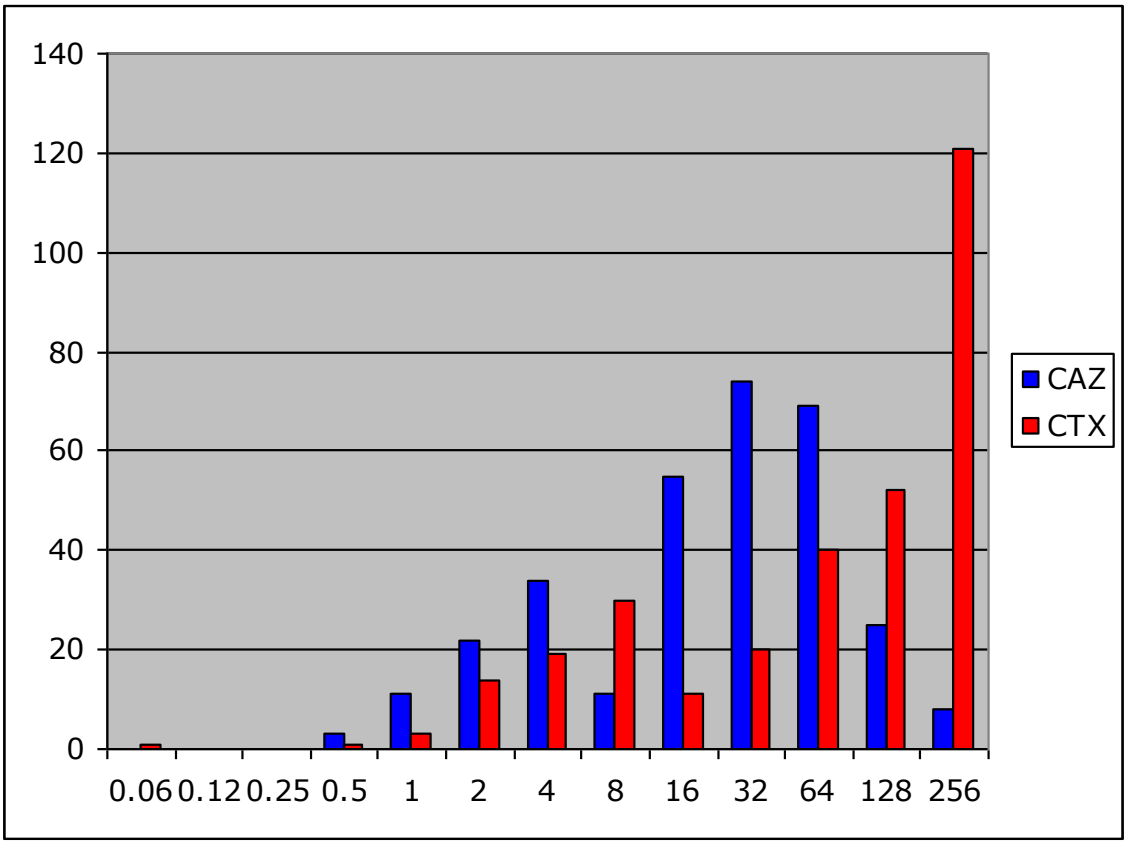


Figure 21: Ceftazidime and cefotaxime MIC range in isolates with CTX-M group 2 genes

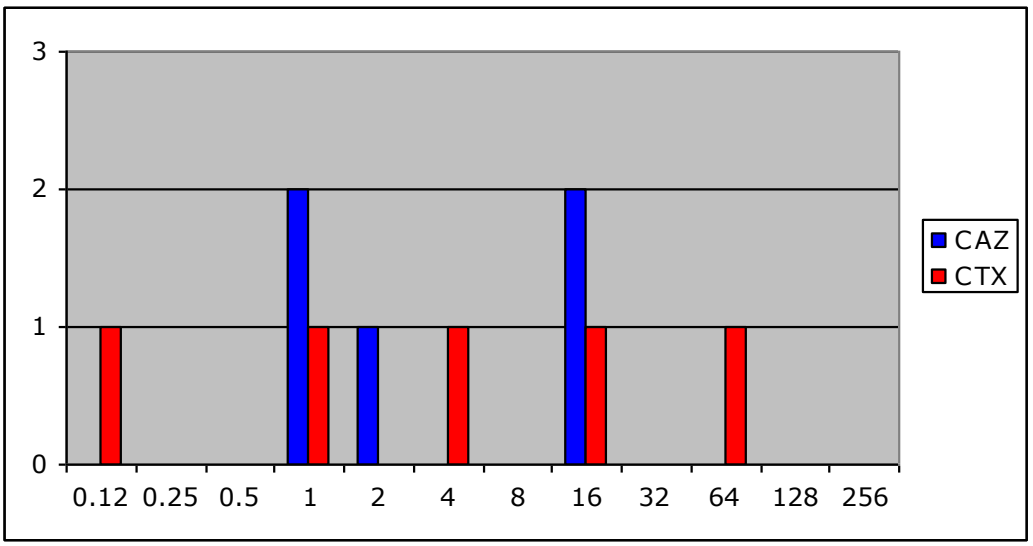


Figure 22: Ceftazidime and cefotaxime MIC range in isolates with CTX-M group 8 genes

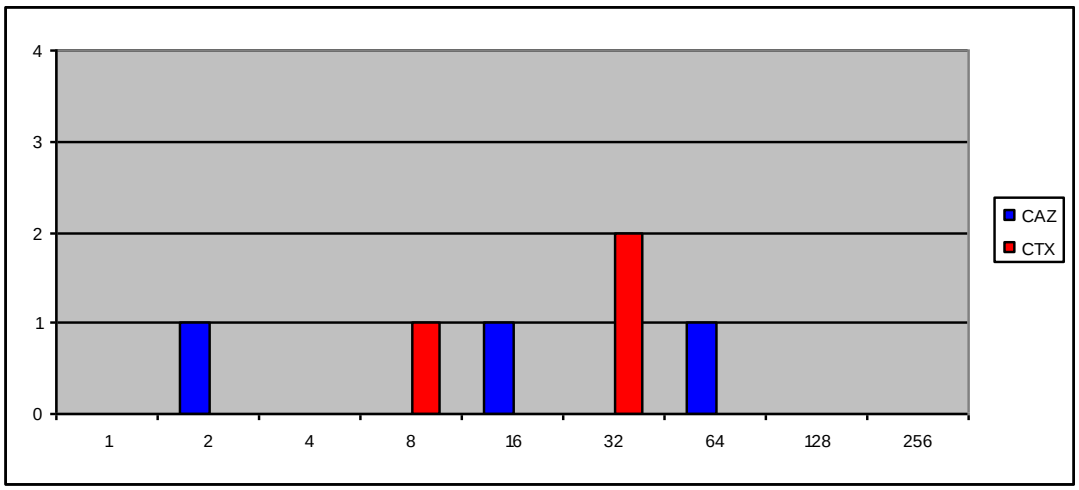


Figure 23: Ceftazidime and cefotaxime MIC range in isolates with CTX-M group 9 genes

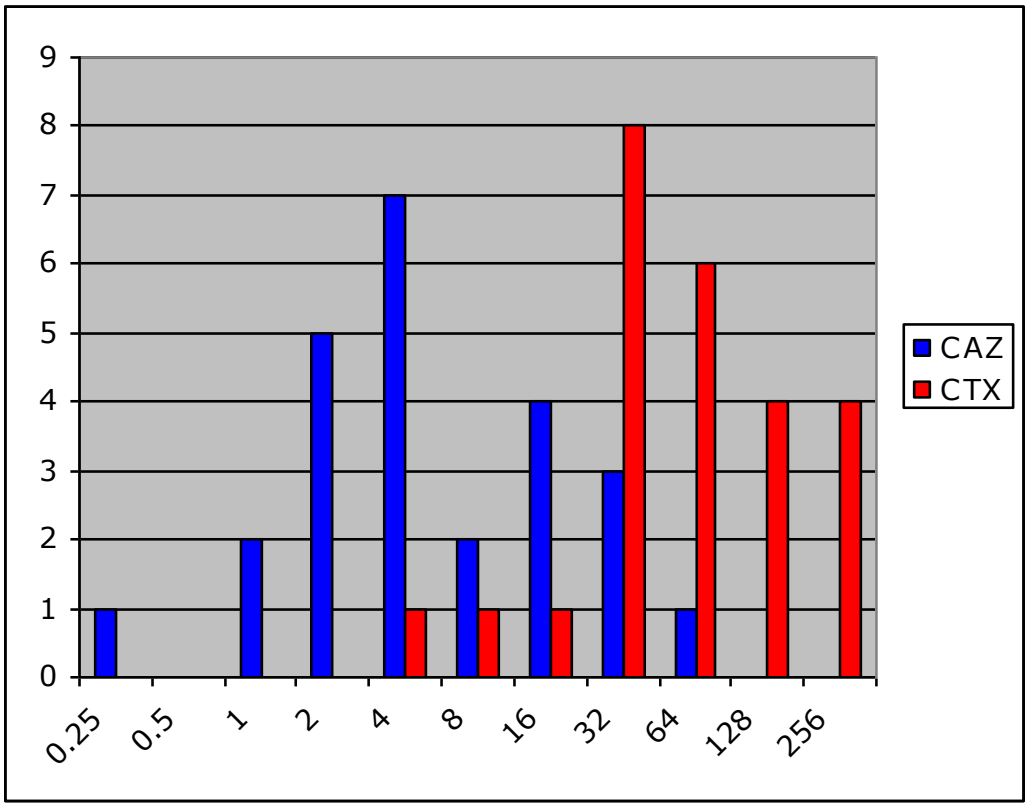
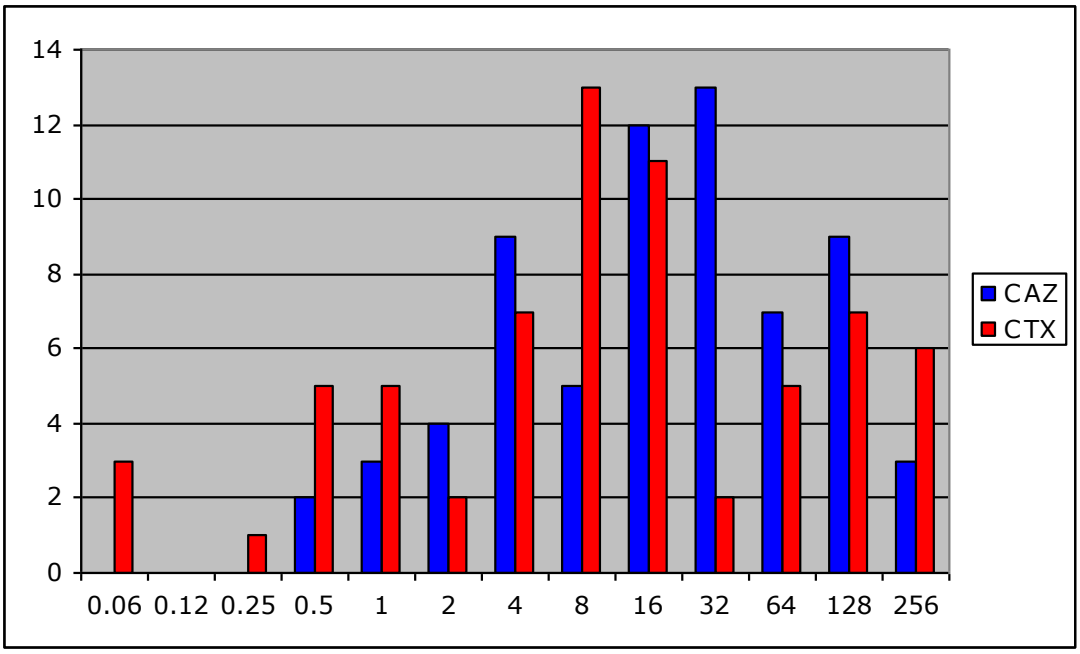


Figure 24: Ceftazidime and cefotaxime MIC range in isolates with non-CTX-M ESBL genes



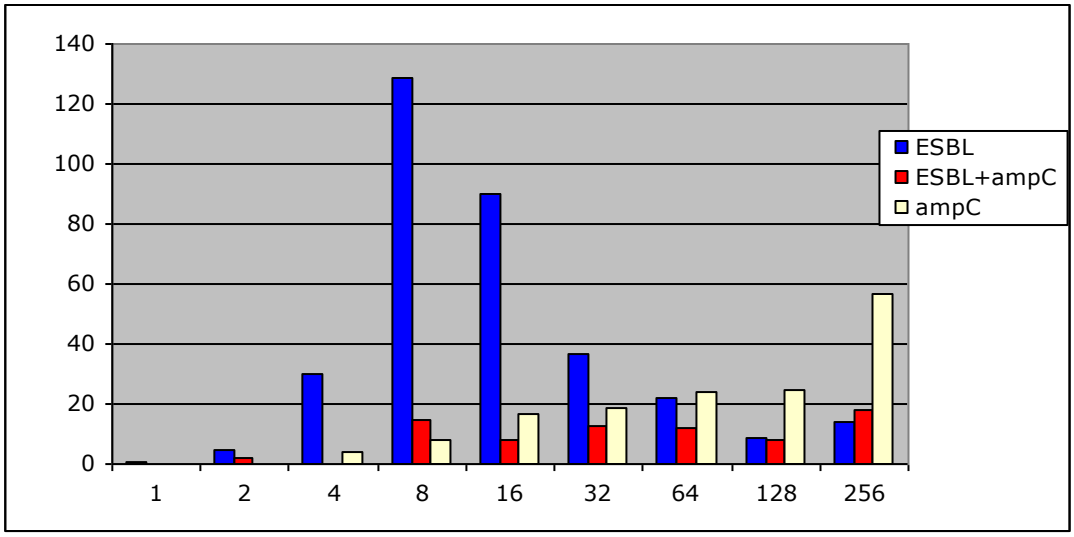
AmpC ß-lactamases

AmpC enzymes are known to hydrolyse cefoxitin far more rapidly than ceftazidime or cefotaxime. The data in this study confirms that ampC enzymes hydrolyse cefoxitin far better than ESBL enzymes (Table 15, Figure 25).

Table 15: Cefoxitin (FOX) MIC range and GeoMeans for various resistance mechanisms

Resistance mechanism	FOX MIC Range (mg/L)	Geo Mean
ESBL	1 - 256	15.0
ESBL + AmpC	2 - 256	44.4
AmpC	4 - 256	78.7

Figure 25: Cefoxitin MIC range in isolates with ampC alone, ESBL alone and ampC in combination with ESBL



In isolates exhibiting either hyper expression of chromosomal ampC enzymes alone or plasmid-mediated ampC enzymes alone, the geometric means for cefoxitin, ceftazidime and cefotaxime decrease respectively. (Table 16, Figures 26 & 27). This data also shows that MICs of cefoxitin, ceftazidime and cefotaxime are higher in isolates harbouring plasmid-mediated ampC enzymes alone when compared to isolates hyper-expressing the chromosomal ampC enzyme.

In isolates exhibiting hyper-expression of native ampC enzymes alone 12.3% were sensitive to ceftazidime, 25.4% were sensitive to cefotaxime and 0% were sensitive to cefoxitin. In isolates harbouring plasmid-mediated ampC enzymes none were sensitive to any cephalosporin tested.

Table 16: Cefoxitin (FOX) MIC range and GeoMeans for isolates containing hyper-expressed chromosomal and plasmid-mediated ampC resistance mechanisms alone

Resistance mechanism	FOX MIC Range (mg/L)	Geo Mean	CAZ MIC Range (mg/L)	Geo Mean	CTX MIC Range (mg/L)	Geo Mean
c-ampC	4 - 256	74.0	0.25 – 256	12.2	0.015 – 256	5.9
p-ampC	64 - 256	122.6	2 - 256	73	2 - 256	14.1

Figure 26: Ceftazidime, cefotaxime and cefoxitin MIC range in isolates with hyper-expressed chromosomal ampC genes

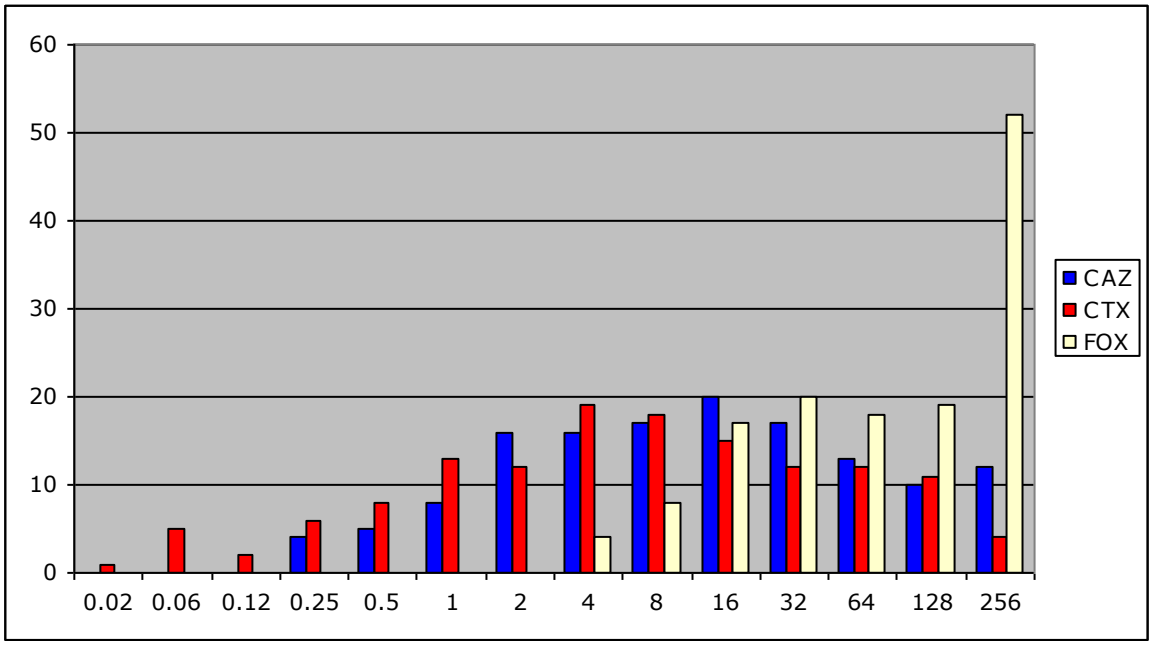
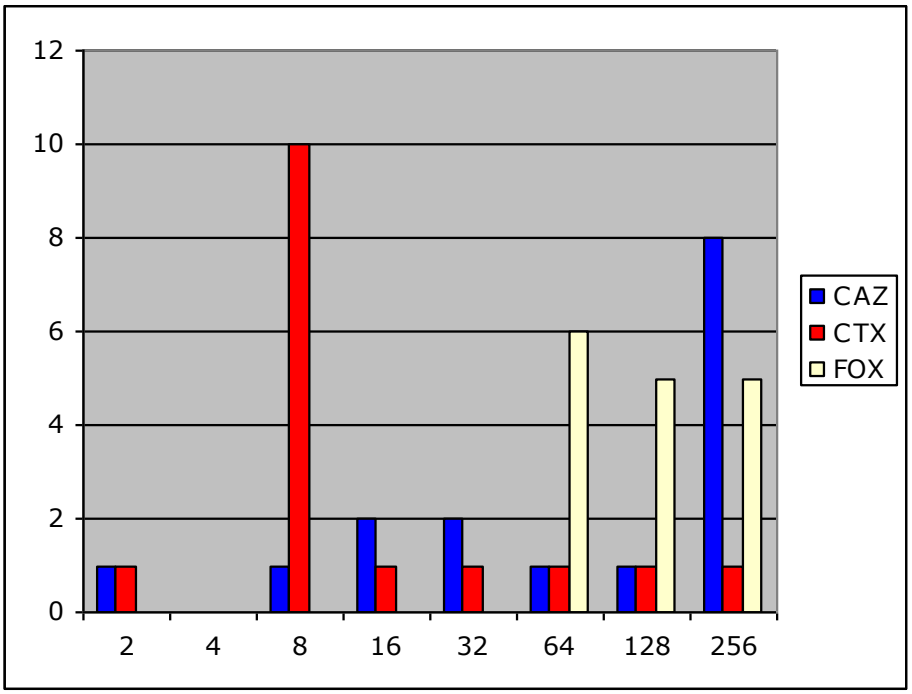


Figure 27: Ceftazidime, cefotaxime and cefoxitin MIC range in isolates with plasmid-mediated ampC genes



3.4.8 Plasmid Mediated AmpC ß-lactamases

Plasmid-mediated ampC β-lactamases were found in 21 (3.6%) out of 580 3GC resistant isolates. In 16 isolates plasmid ampC β-lactamases were found alone compared to 5 isolates where they were found in combination with other resistance mechanisms. Of all isolates exhibiting an ampC β-lactamase phenotype (230), 9.1% harboured plasmid-mediated ampC β-lactamases and 90.9% exhibited hyper-expression of chromosomal ampC β-lactamases.

Plasmid mediated ampC β-lactamases were found predominantly alone (in 17 isolates) and only in *E. coli* and *K. pneumoniae.* In 4 isolates plasmid-mediated ampC β-lactamases were found in combination with SHV (1 isolate) and CTX-M genes (3 isolates).

The most common enzyme found in Wales, as in the UK was CIT, a CMY type enzyme. In a recent UK report 8 out of the 11 isolates harbouring FOX enzymes were isolated from a centre in Wales. In this study two isolates contained FOX enzymes, which originate from *Aeromonas spp*.. The second most common enzyme found in Welsh isolates was EBC, originating from *Enterobacter cloacae* (Table 17), an enzyme rarely found in isolates from the UK.

Table 17: Breakdown of plasmid-mediated ampC genes found in Welsh Enterobacteriaceae

Laboratory	Total No.	Origin	No	ID	Other mechanism involved	Type
Newport	2	GP	2	EC	None	CIT
Cardiff	12	GP	7	EC	None	CIT
		H	2	EC	None	CIT
		GP	1	EC	SHV	FOX
		GP	1	EC	CTX-M (9)	CIT
		GP	1	KPN	CTX-M (1)	FOX
Llantrisant	1	H	1	KPN	None	EBC
Rhyl	3	H	1	KPN	None	EBC
		GP	2	KPN	None	EBC
Aberystwyth	1	GP	1	EC	None	CIT
Bangor	1	H	1	EC	CTX-M (1)	CIT
	1	H	1	KPN	CTX-M (8)	CIT

The prevalence of isolates containing a plasmid-mediated β-lactamase per Laboratory is shown in Table 18. Cardiff has the highest overall prevalence; 8.2% of all 3rd generation cephalosporin resistant isolates

harboured plasmid-mediated β-lactamases. The percentages of isolates containing plasmid mediated ampC β-lactamases in isolates exhibiting an ampC phenotype, in *E. coli* and in *Klebsiella spp.* at each laboratory are also shown.

Of the isolates containing plasmid-mediated ampC ß-lactamases, 15 were *E. coli* and 6 *K. pneumoniae*. At Rhyl and Llantrissant only *K. pneumoniae* were found to harbour these β-lactamases, whereas in Aberystwyth and Newport only *E. coli* were affected. In a recent study from ARMRL, 49% of *E. coli* and 55% of *Klebsiella spp.* sent for cephalosporin resistance confirmation and exhibiting an ampC phenotype were positive for plasmid-mediated ampC β-lactamases. In Wales 230 isolates exhibited an ampC phenotype, with 21 (9.1%) harbouring plasmid-mediated ampC β-lactamases. In contrast, at Cardiff, 22.6% of isolates exhibiting an ampC phenotype harboured a plasmid-mediated β-lactamase, with the other Laboratory prevalence between 3% and 10% (Table 18).

(Ref: Woodford et al JAC 2007, 59:102-5.)

Table 18: Percentage of *E. coli* and *Klebsiella* species harbouring plasmid-mediated ampC β-lactamases per laboratory

Laboratory	Total isolates with 3GCR	% with p-ampC	No strains with ampC	% with p-ampC	No of EC	% with p-ampC	No of Klebsiella	% with p-ampC
Newport	116	1.7%	56	3.6%	43	4.6%	26	0%
Cardiff	146	8.2%	53	22.6%	92	13%	21	4.8%
Llantrisant	41	2.4%	28	3.6%	29	0%	6	16.7%
Rhyl	80	3.8%	33	9.1%	45	0%	11	27.3%
Aberystwyth	25	4%	11	9.1%	17	5.9%	1	0%
Bangor	77	2.5%	16	6.3%	61	1.6%	2	50%

3.4.9 Multi-Resistance in 3rd generation cephalosporin resistant Enterobacteriaceae

Ciprofloxacin

Most Enterobacteriaceae with CTX-M or non-CTX-M ESBLs were also resistant to ciprofloxacin. 92.4% of isolates containing CTX-M alone were also ciprofloxacin resistant, including 91.2% and 94.9% of *E. coli* and Klebsiella isolates. Ciprofloxacin resistance was seen in over 50% of isolates containing other resistance mechanisms. The geometric mean for ciprofloxacin was higher for CTX-M containing isolates than for isolates with other resistance mechanisms (Figure 28-30, Table 19-21).

The prevalence of ciprofloxacin resistance was between 61.7% and 100% in isolates from Welsh laboratories.

Figure 28: Resistance to ciprofloxacin in isolates with various resistance mechanisms

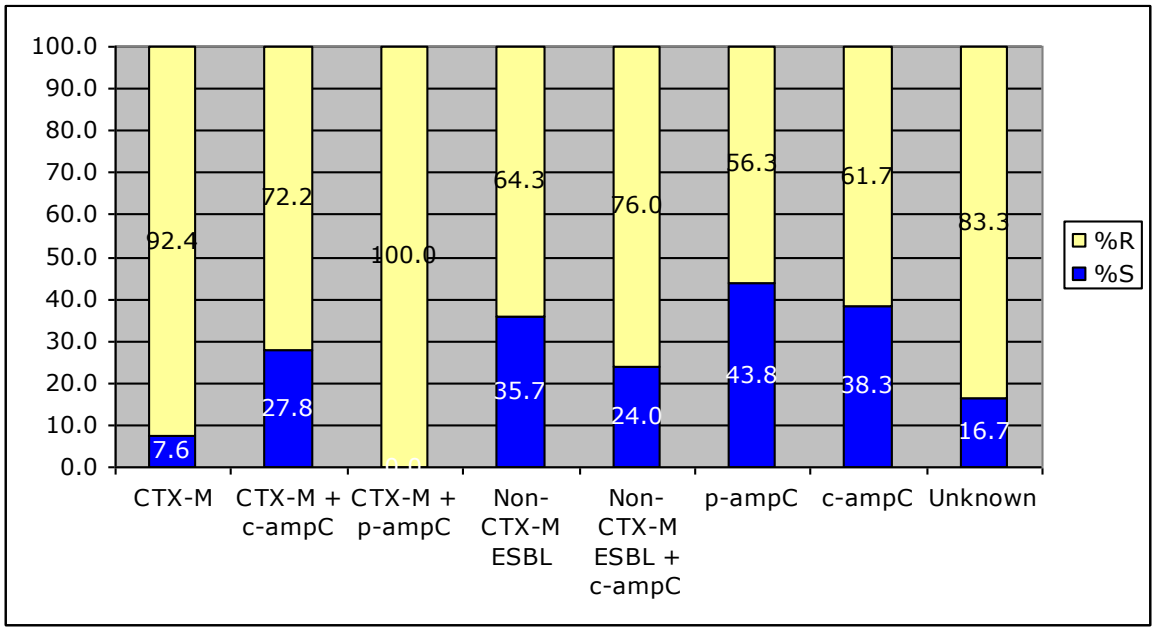


Table 19: Ciprofloxacin GeoMean MICs in isolates with various resistance mechanisms

Resistance Mechanism	GeoMean MIC
CTX-M	62.7
CTX-M + c-ampC	10.2
CTX-M + p-ampC	16
Non-CTX-M ESBL	2.6
Non-CTX-M ESBL + ampC	23.9
p-ampC	1.7
c-ampC	1.3
Unknown	15.1

Figure 29: Resistance to ciprofloxacin in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

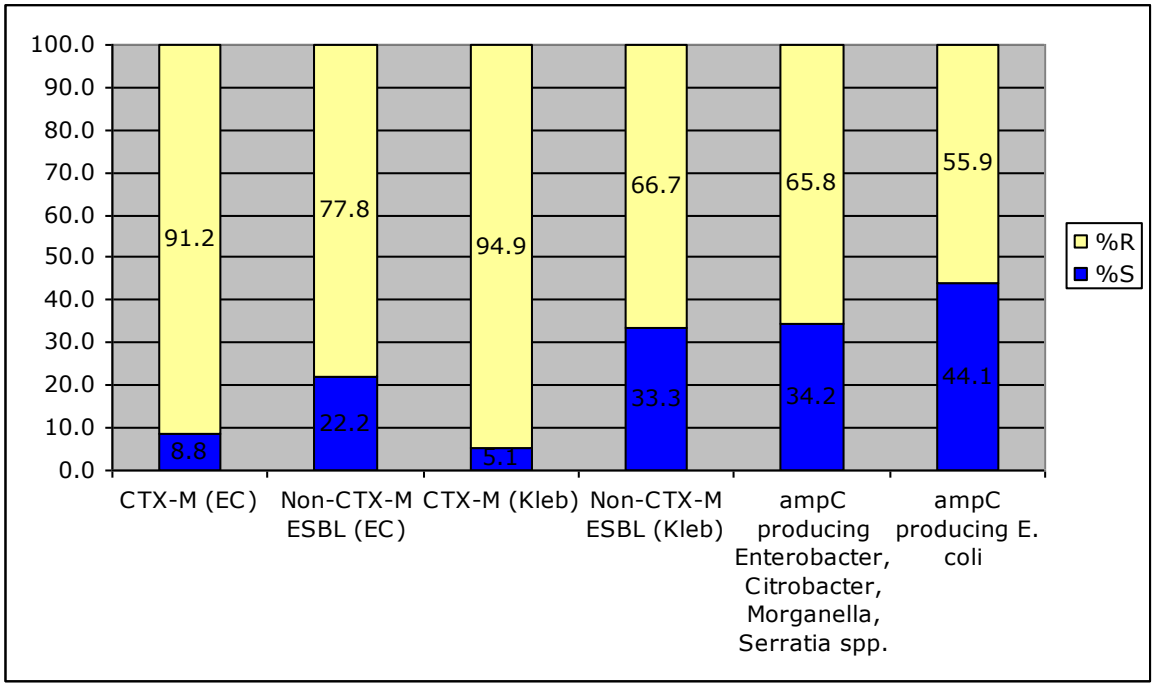


Table 20: Ciprofloxacin GeoMean MICs in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

Resistance Mechanism	GeoMean MIC
CTX-M (EC)	64.3
Non-CTX-M ESBL (EC)	7.9
CTX-M (Kleb)	38.2
Non-CTX-M ESBL (Kleb)	2.9
ampC (Enterobacter spp.)	1.3
ampC (EC)	1.3

Figure 30: Ciprofloxacin resistance per laboratory

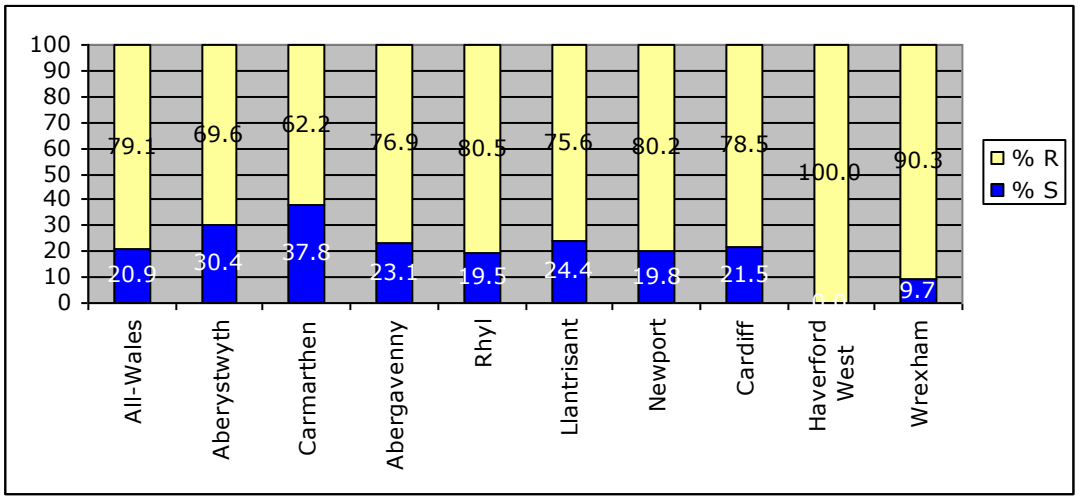


Table 21: Ciprofloxacin GeoMean MICs per laboratory

Laboratory	GeoMean
All-Wales	12.04
Aberystwyth	11.38
Carmarthen	2.87
Abergavenny	6.75
Rhyl	9.79
Llantrisant	7.93
Newport	10.63
Cardiff	13.87
Haverfordwest	54.86
Wrexham	48.83

Gentamicin

32.8% of isolates containing CTX-M alone were also gentamicin resistant, including 25.4% and 11.1% of *E. coli* and Klebsiella isolates. Gentamicin resistance was mostly seen in isolates with unknown resistance mechanisms. The geometric mean for gentamicin was higher for CTX-M containing isolates than for isolates with other specific resistance mechanisms (Figure 31-33, Table 22-24).

The prevalence of gentamicin resistance was between 14.6% and 77.8% in isolates from Welsh laboratories.

Figure 31: Resistance to gentamicin in isolates with various resistance mechanisms

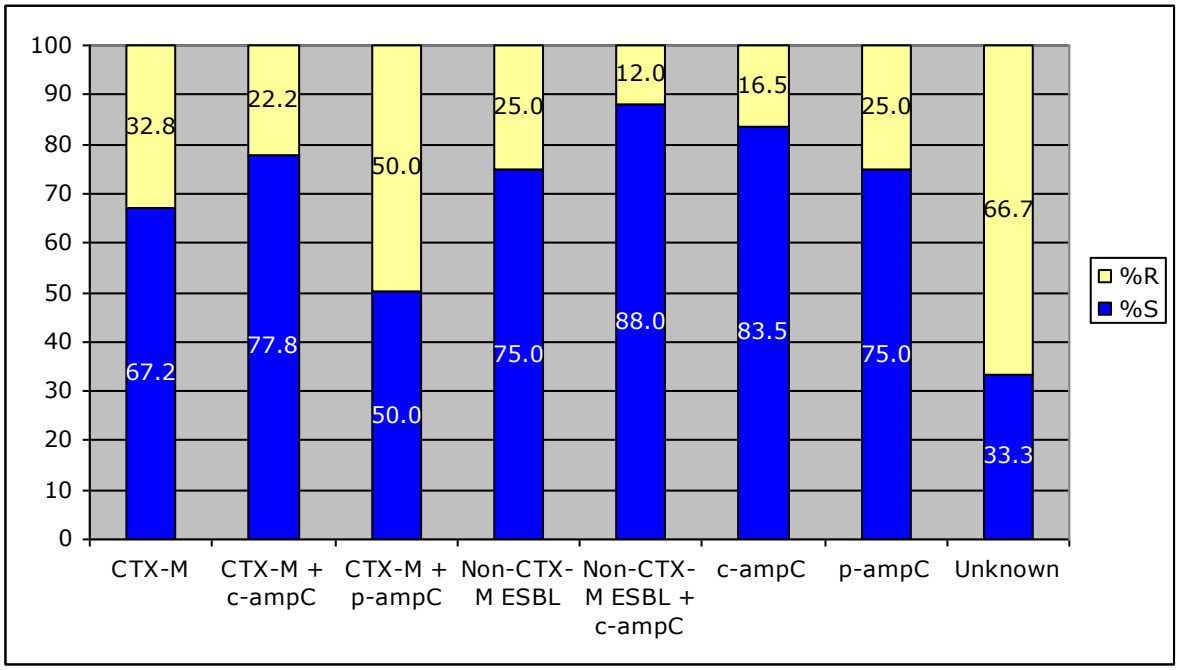


Table 22: Gentamicin GeoMean MICs in isolates with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M	2.6
CTX-M + c-ampC	1.4
CTX-M + p-ampC	4
Non-CTX-M ESBL	1.4
Non-CTX-M ESBL + ampC	1.1
p-ampC	0.9
c-ampC	1.5
Unknown	4.3

Figure 32: Resistance to gentamicin in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

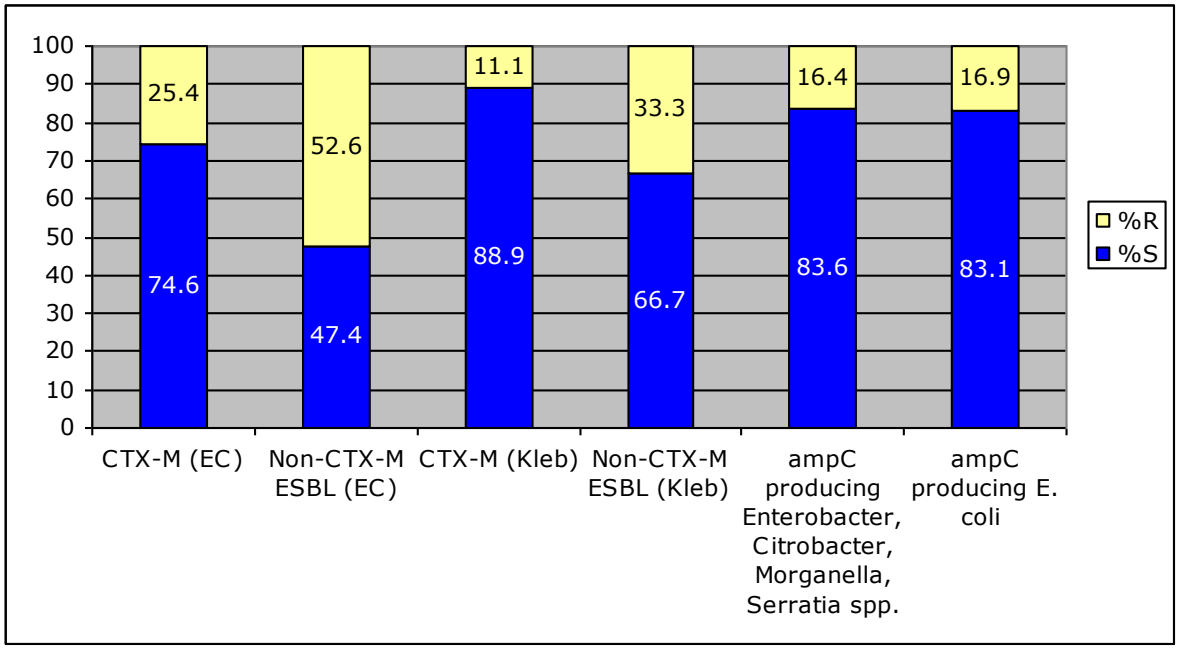


Table 23: Gentamicin GeoMean MICs in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M (EC)	1.95
Non-CTX-M ESBL (EC)	5.25
CTX-M (Kleb)	0.73
Non-CTX-M ESBL (Kleb)	1.82
ampC (Enterobacter spp.)	0.89
ampC (EC)	0.95

Figure 33: Gentamicin resistance per laboratory

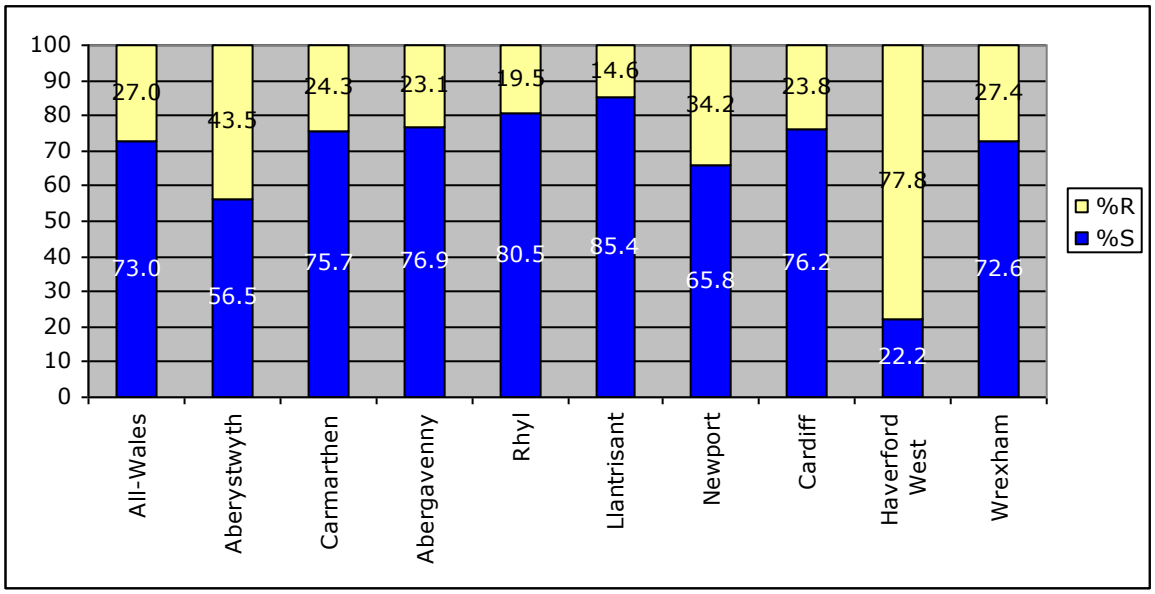


Table 24: Gentamicin GeoMean MICs per laboratory

Laboratory	GeoMean
All-Wales	1.78
Aberystwyth	3.77
Carmarthen	1.72
Abergavenny	1.17
Rhyl	1.22
Llantrisant	0.87
Newport	2.30
Cardiff	1.47
Haverfordwest	18.38
Wrexham	2.65

Nitrofurantoin

Nitrofurantoin resistance was seen predominantly in non CTX-M containing isolates, with 28.0% and 43.5% of isolates containing CTX-M and non-CTX-M being nitrofurantoin resistant. Nitrofurantoin resistance was more prevalent in Klebsiella isolates compared to *E. coli*. The geometric mean for gentamicin was higher for non-CTX-M containing isolates than for isolates with CTX-M (Figures 34-36, Tables 25-27).

The prevalence of nitrofurantoin resistance was between 23.1% and 56.8% in isolates from Welsh laboratories.

Figure 34: Resistance to nitrofurantoin in isolates with various resistance mechanisms

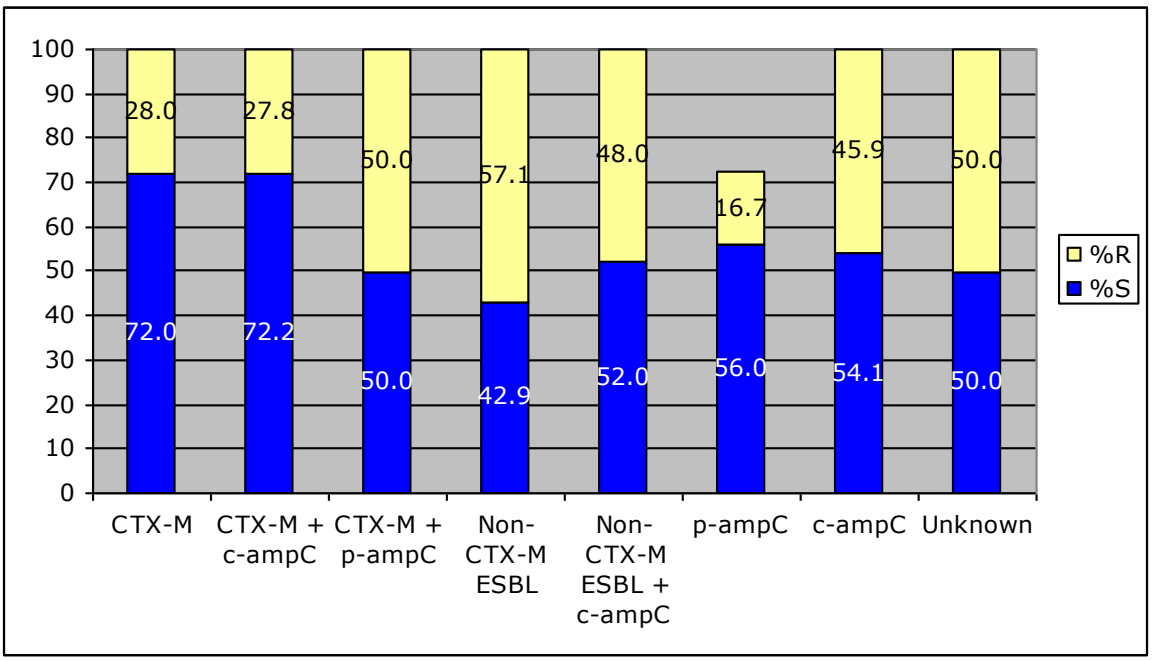


Table 25: Nitrofurantoin GeoMean MICs in isolates with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M	18.9
CTX-M + c-ampC	13.5
CTX-M + p-ampC	45.3
Non-CTX-M ESBL	43.1
Non-CTX-M ESBL + ampC	40.6
p-ampC	14.7
c-ampC	29.9
Unknown	40.3

Figure 35: Resistance to nitrofurantoin in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

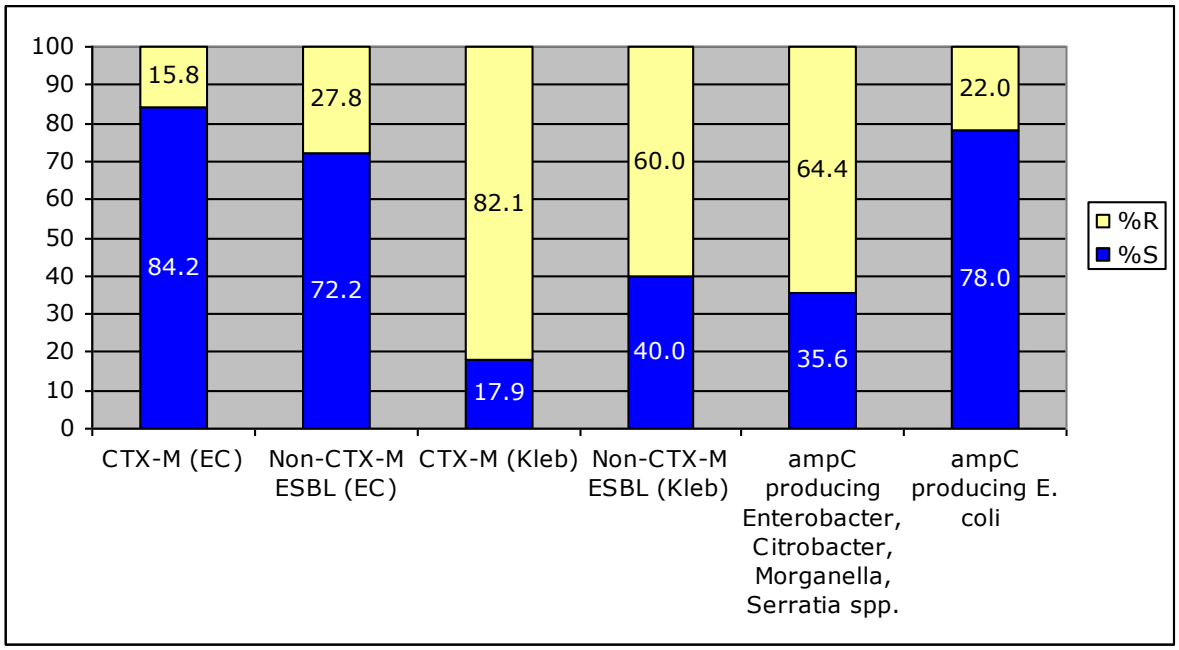


Table 26: Nitrofurantoin GeoMean MICs in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M (EC)	13.7
Non-CTX-M ESBL (EC)	21.8
CTX-M (Kleb)	77.8
Non-CTX-M ESBL (Kleb)	48.5
ampC (Enterobacter spp.)	46.3
ampC (EC)	17.2

Figure 65: Nitrofurantoin resistance per laboratory

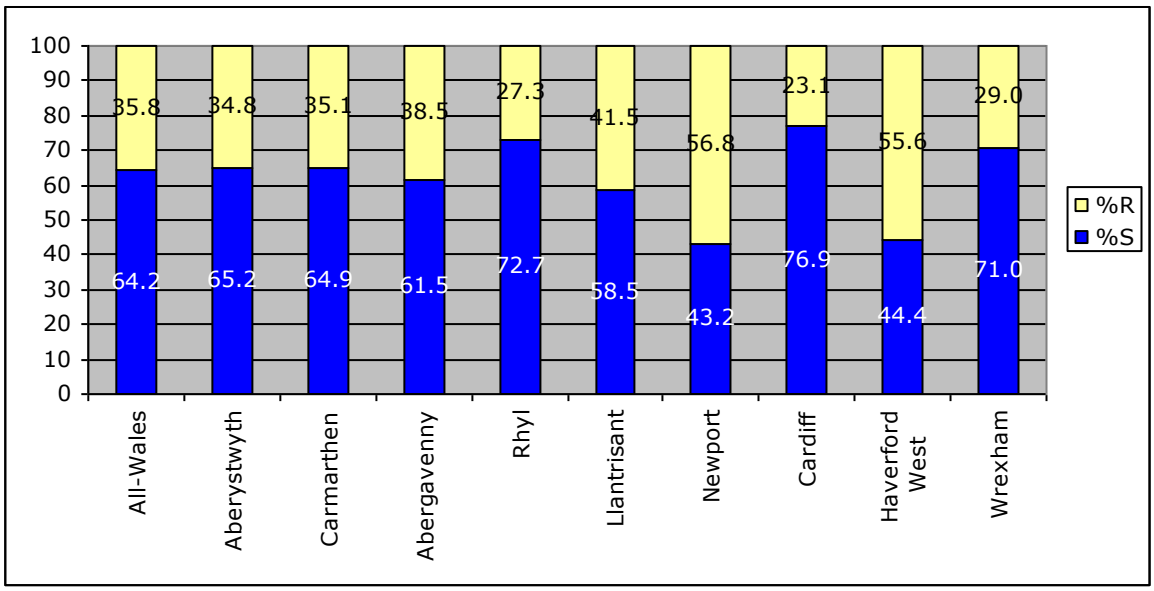


Table 27: Nitrofurantoin GeoMean MICs per laboratory

Laboratory	GeoMean
All-Wales	23.06
Aberystwyth	22.29
Carmarthen	22.00
Abergavenny	22.03
Rhyl	18.48
Llantrisant	24.83
Newport	38.59
Cardiff	15.41
Haverfordwest	40.32
Wrexham	26.17

Mecillinam

Most isolates showed susceptibility to mecillinam. Resistance was seen predominantly in isolates with unknown resistance mechanisms and in 39.3% of isolates containing ESBL other than CTX-M. Mecillinam resistance was more prevalent in Klebsiella isolates compared to *E. coli*, with 53.33% resistant. (Figures 37-39, Tables 28-30).

The prevalence of mecillinam resistance was between 4.8% and 55.6% in isolates from Welsh laboratories.

Figure 37: Resistance to mecillinam in isolates with various resistance mechanisms

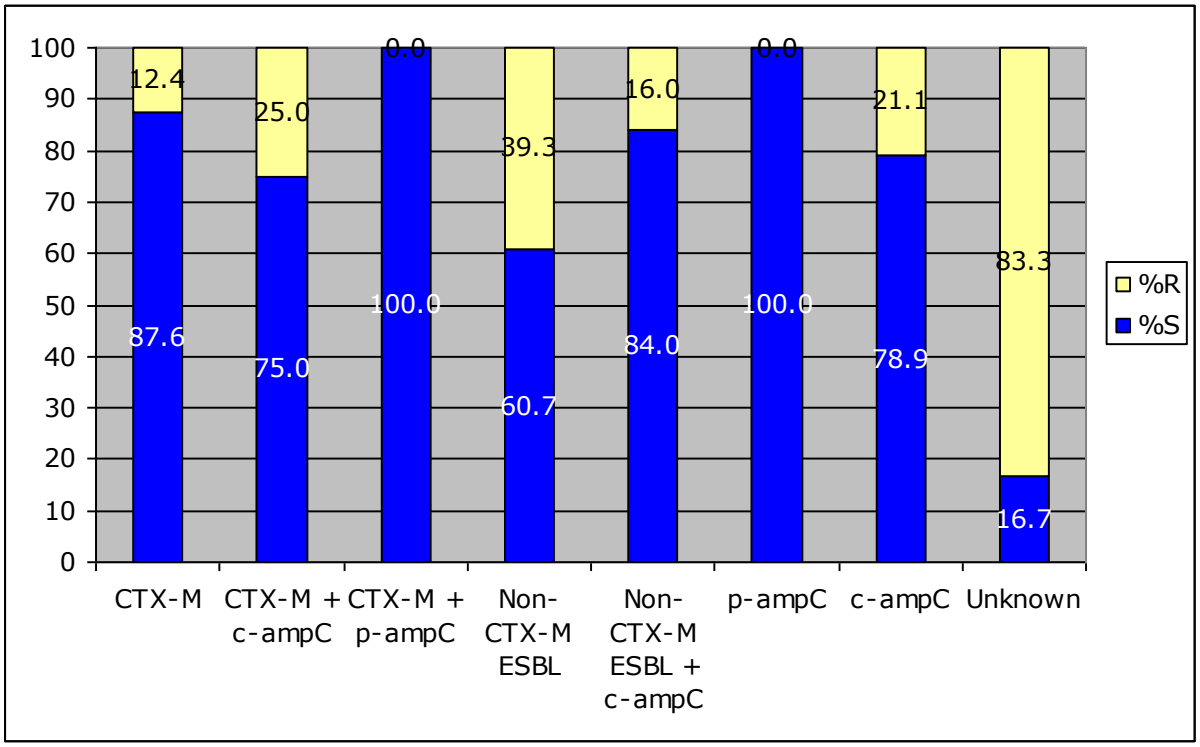


Table 28: Mecillinam GeoMean MICs in isolates with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M	1.65
CTX-M + c-ampC	2.87
CTX-M + p-ampC	2.00
Non-CTX-M ESBL	9.09
Non-CTX-M ESBL + ampC	2.49
p-ampC	0.75
c-ampC	2.04
Unknown	76.11

Figure 38: Resistance to mecillinam in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

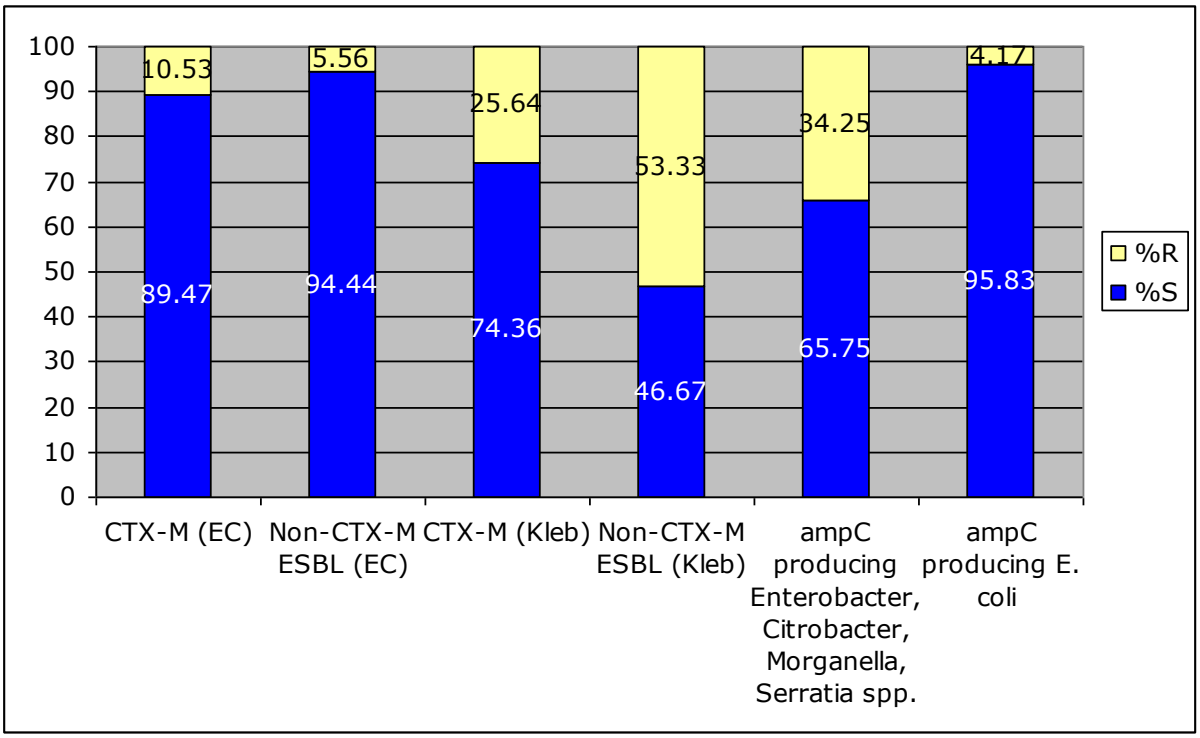


Table 29: Mecillinam GeoMean MICs in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M (EC)	2.12
Non-CTX-M ESBL (EC)	1.21
CTX-M (Kleb)	6.24
Non-CTX-M ESBL (Kleb)	11.58
ampC (Enterobacter spp.)	4.07
ampC (EC)	0.89

Figure 39: Mecillinam resistance per laboratory

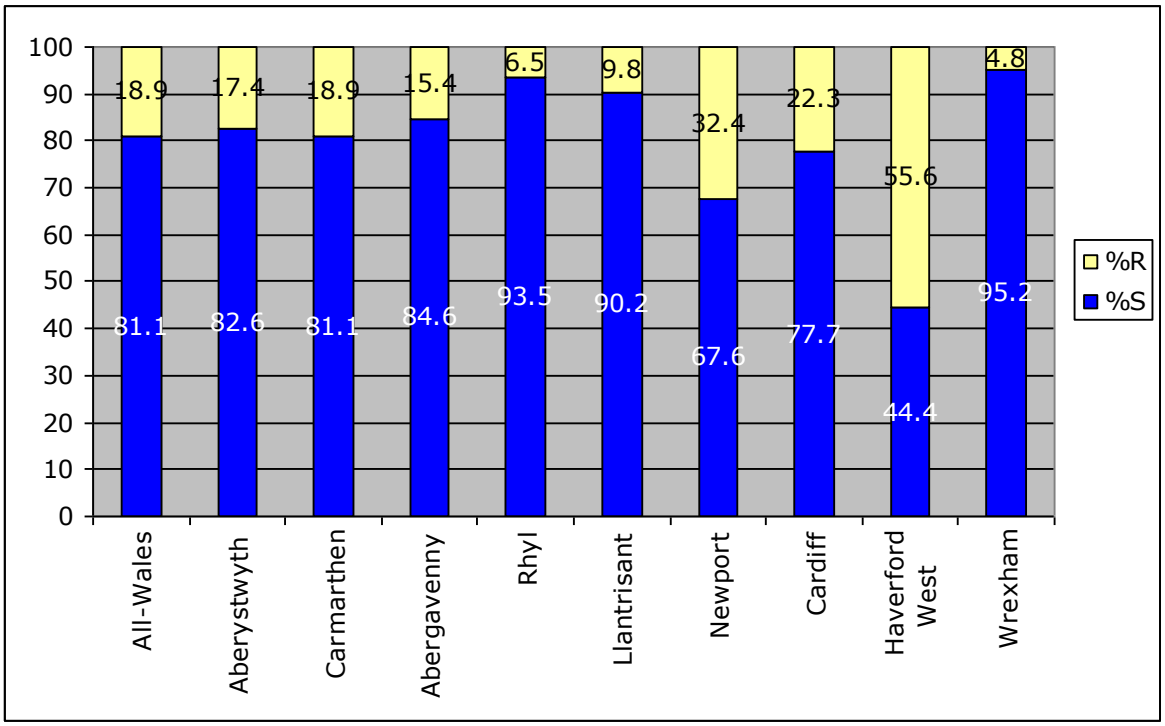


Table 30: Mecillinam GeoMean MICs per laboratory

Laboratory	GeoMean
All-Wales	2.7
Aberystwyth	2.14
Carmarthen	1.88
Abergavenny	2.00
Rhyl	1.48
Llantrisant	1.16
Newport	4.74
Cardiff	2.26
Haverfordwest	29.63
Wrexham	1.13

Temocillin

Most isolates showed susceptibility to temocillin, except isolates containing CTX-M plus plasmid-mediated ampC ß-lactamases. Geometric means of isolates containing CTX-M enzymes were higher than for those isolates harbouring other resistance mechanisms. Resistance rate was also high in isolates with unknown resistance mechanisms. (Figures 40-42, Tables 31-33).

The prevalence of temocillin resistance was between 0% and 46.9% in isolates from Welsh laboratories.

Figure 40: Resistance to temocillin in isolates with various resistance mechanisms

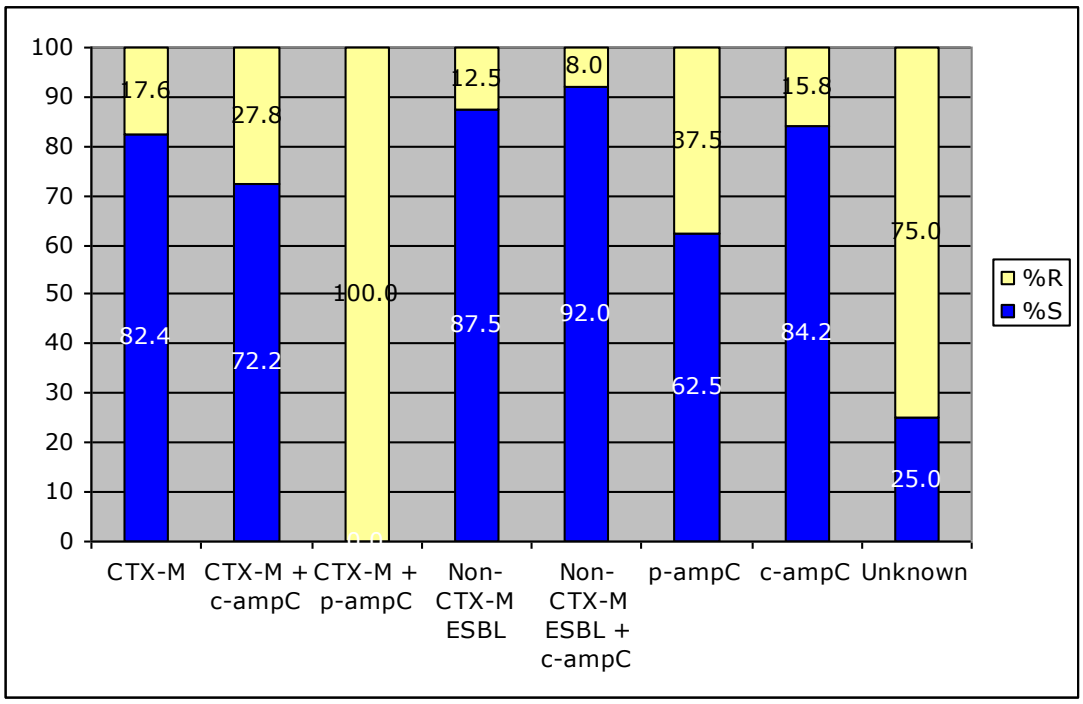


Table 31: Temocillin GeoMean MICs in isolates with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M	3.1
CTX-M + c-ampC	4.08
CTX-M + p-ampC	32
Non-CTX-M ESBL	1.58
Non-CTX-M ESBL + ampC	1.45
p-ampC	7.03
c-ampC	2.62
Unknown	40.18

Figure 41: Resistance to temocillin in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

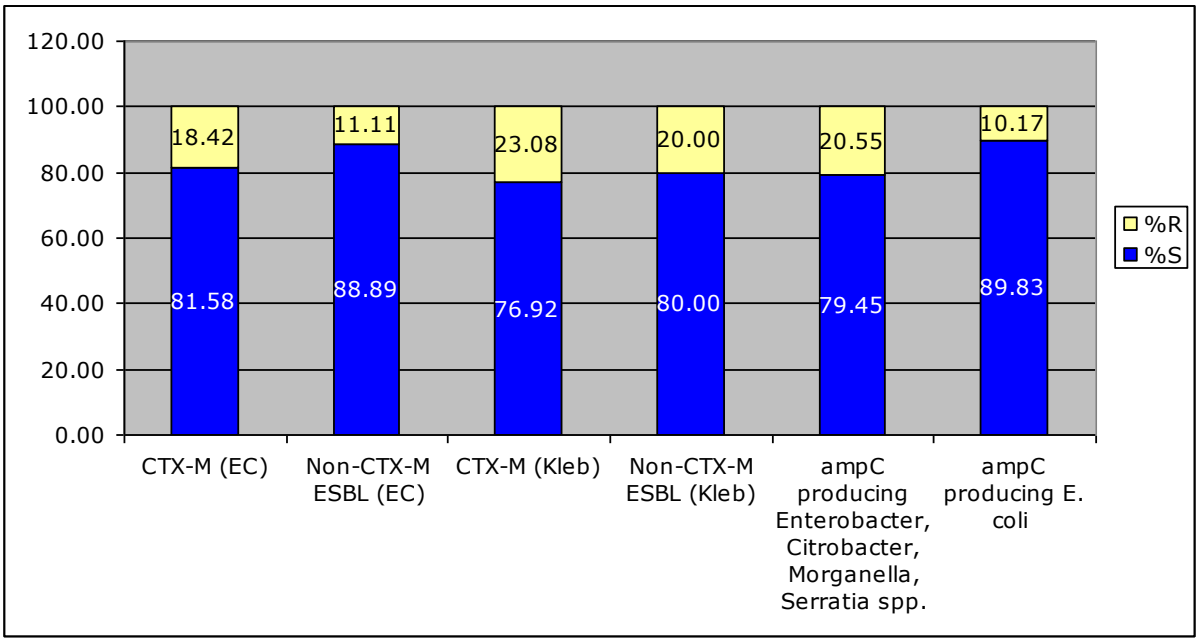


Table 32: Temocillin GeoMean MICs in *E. coli*, *Klebsiella* and *Enterobacter* species with various resistance mechanisms

Resistance Mechanism	GeoMean
CTX-M (EC)	3.01
Non-CTX-M ESBL (EC)	1.17
CTX-M (Kleb)	2.85
Non-CTX-M ESBL (Kleb)	2.52
ampC (Enterobacter spp.)	2.78
ampC (EC)	2.41

Figure 42: Temocillin resistance per laboratory

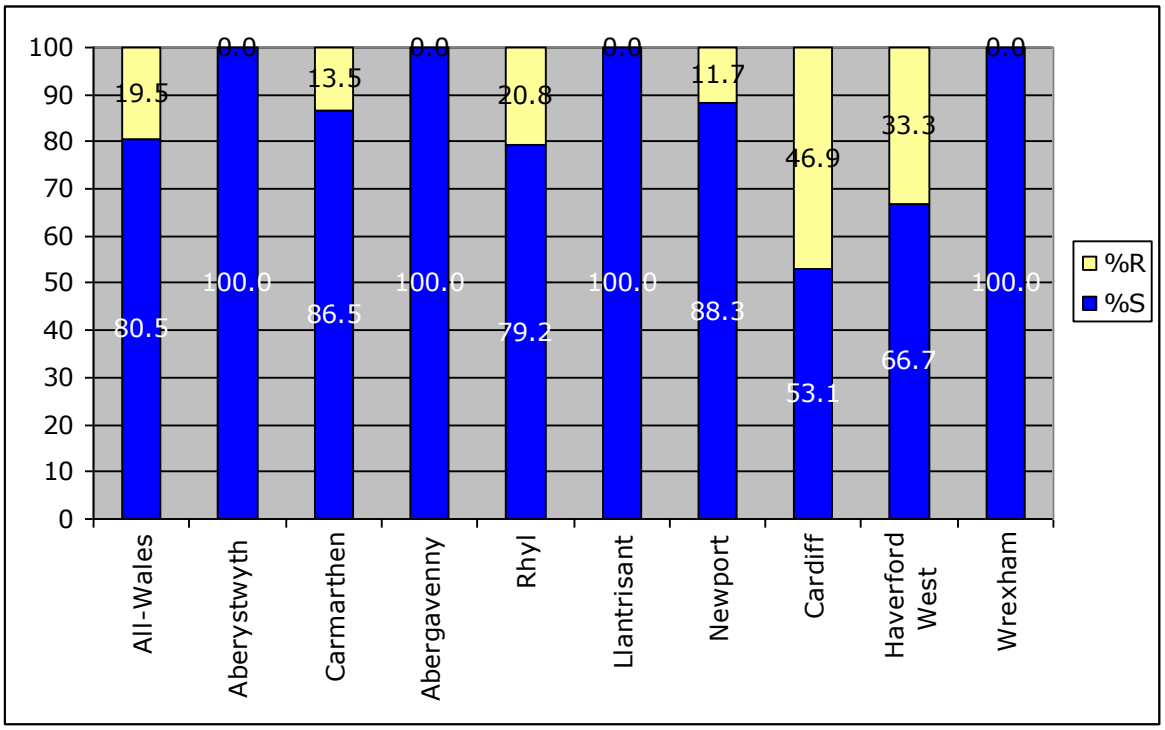


Table 33: Temocillin GeoMean MICs per laboratory

Laboratory	GeoMean
All-Wales	2.95
Aberystwyth	0.86
Carmarthen	2.04
Abergavenny	0.68
Rhyl	5.38
Llantrisant	0.95
Newport	1.22
Cardiff	16.26
Haverfordwest	7.41
Wrexham	0.87

This data confirms the association between multi-resistance and ESBL presence due to the transfer of multi-resistant plasmids.