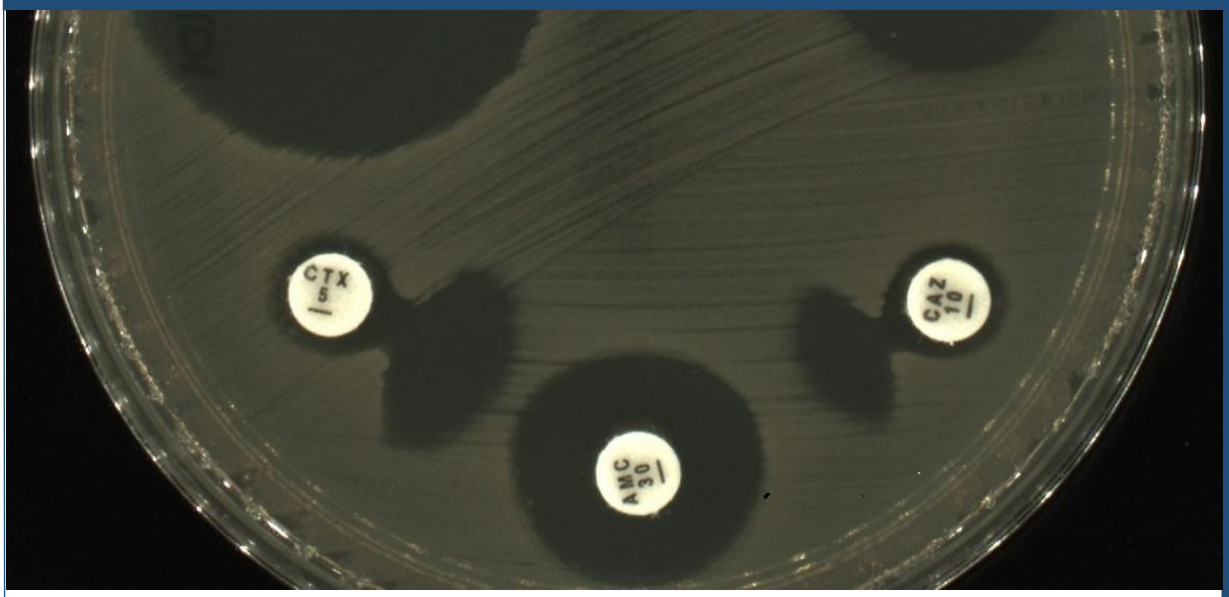

Targeted Surveillance of 3rd Generation Cephalosporin Resistance in Enterobacterales

Report for 2014 surveillance:

All Wales, including data from 2008 report

Specialist Antimicrobial Chemotherapy Unit

Published: 6th October 2020

The Healthcare Associated Infection and Antimicrobial Resistance Programme can be accessed via the Public Health Wales website at: http://www.publichealthwales.org.

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CONTENTS

1 Introduction

Antimicrobial resistance is an increasing problem both in hospital and community settings, leading to reduced clinical therapy options and poor patient outcomes. Resistance in Gram-negative bacteria, particularly carbapenem resistance, is the most important clinically. However, resistance to 3rd generation cephalosporins in Enterobacterales is a long standing problem and drives carbapenem use within hospitals. 3rd generation cephalosporin resistance is most commonly caused by CTX-M Extended Spectrum β-lactamases in *E. coli* and hyper-expression of chromosomal ampC β-lactamases in other Enterobacterales. Less common causes of 3rd generation cephalosporin resistance but important for infection control purposes are plasmidic ampC-type β-lactamases, found mainly in *E. coli* and *K. pneumoniae*. In Wales all coliforms from urines are screened for susceptibility to cefpodoxime, whilst cefotaxime and ceftazidime are employed for Enterobacterales from other clinical samples. In urinary coliforms, approximately 6% from the community and 12% from hospitals are resistant to cefpodoxime respectively.

In 2008, the first 3rd generation cephalosporin resistance (3GCR) targeted surveillance study was performed to provide an overview of molecular mechanisms responsible for 3rd generation cephalosporin resistance in Wales. The long term trend of these resistance mechanisms will be analysed by performing the surveillance every 5 years.

This report details the 2014 data and comparative 2008 data.

2 Methods

2.1 Strains

In Wales approximately 90,000 hospital and community coliforms are isolated per year, approximately 4500 (5%) of these isolates are cefpodoxime resistant, and of these 3600 (80%) contain Extended Spectrum β-lactamases (ESBLs). Based upon these assumptions and in order to detect any year-on-year increase in resistance, 1/6th of cefpodoxime resistant *Enterobacterales* were requested from Microbiology laboratories across Wales (Table 1).

Table 1: Numbers of isolates requested; 1/6th cefpodoxime resistant coliforms

Hospital Location	No Req'd	Hospital E. coli	Hospital Coliform	GP E. coli	GP Coliform
Bronglais Hospital, Aberystwyth	26	5	3	14	4
Royal Gwent, Newport	135	24	47	32	32
UHW, Cardiff	147	41	24	62	20
Singleton, Swansea	196	19	81	20	76
WWGH, Carmarthen	112	22	26	33	31
Royal Glamorgan, Llantrisant	41	12	8	17	4
Ysbyty Glan Clwyd, Rhyl	230	53	39	96	42
Total	887	176	228	274	209

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

2.2 Phenotypic Characterisation

Minimum Inhibitory Concentration (MIC) Determination

MICs were determined by broth microdilution (BMD) using the international standard method (ISO 201776-1) for ciprofloxacin (Pharmacy), temocillin (Eumedica), mecillinam (Sigma-Aldrich), nitrofurantoin (Sigma-Aldrich), gentamicin (Sigma-Aldrich), cefoxitin (Sigma-Aldrich), cefotaxime (Sigma-Aldrich), ceftazidime (Sigma-Aldrich), meropenem (Pharmacy) and colistin (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 (Sigma-Aldrich) or 200mg/L cloxacillin sodium salt monohydrate (Sigma-Aldrich) to detect ESBL and ampC β-lactamases. Microtitre plates were incubated in air at 35-37°C for 18hrs.[1]

Analysis of Phenotypic Data

MICs were recorded as the lowest antimicrobial concentration where growth of the isolate was inhibited. MICs were interpreted using the current EUCAST clinical breakpoints (version 9 2019).

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 β-lactamases. 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 categorised as RESISTANT (R) to a 3rd generation cephalosporin, and exhibiting ESBL and/or ampC positive phenotypes were confirmed using genotypic methods. Isolates categorised as Susceptible, Increased exposure (I) will be tested alongside the resistant isolates but results will be reported in section 3.7. Isolates confirmed as susceptible to 3rd generation cephalosporins will be omitted from the study.

2.3 Genotypic Characterisation

Real time - Polymerase Chain Reaction (RT-PCR)

Qualitative detection of the most common ESBLs (CTX-M, TEM, SHV and GES) was performed by real-time PCR using PowerUp SYBR Green Master Mix (Life Technologies) in combination with the Quant Studio 6-Flex platform (ABI). An internal control assay targeting the green fluorescent protein (*gfp*) from *Aequorea Victoria* (Accession M62653.1) carried on a bespoke plasmid, is simultaneously run to discount PCR inhibition. Melting curve analysis is applied to check specificity of the PCR amplicons.

E. coli and *K. pneumoniae* isolates exhibiting *ampC* phenotypes were screened for plasmid *ampC* genes (*bla_{ACC}, bla_{MOX}, bla_{EBC}, bla_{FOX}, bla_{CIT}, and bla_{DHA}*) by a multiplex block-based PCR assay and specific primers (Table 2) then imaged using a 1% agarose gel containing Ethidium bromide and UVP GelDoc-XR™ Imaging System.[2]

Table 2: Primer sequences for plasmid ampC assay

Name	Forward Primer Sequence	Reverse Primer Sequence
ACC	AACAGCCTCAGCAGCCGCTAA	TTCGCCGCAATCATCCCTAGC
MOX	GCTGCTCAAGGAGCACAGGAT	CACATTGACATAGGTGTGGTGC
EBC	TCGGTAAAGCCGATGTTGCGG	CTTCCACTGCGGCTGCCAGTT
FOX	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG
CIT	TGGCCAGAACTGACAGGCAAA	TTTCTCCTGAACGTGGCTGGC
DHA	CCGTACGCATACTGGCTTTGC	AACTTTCACAGGTGTGCTGGGT

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

Throughout 2014, 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. Participating laboratories were: West Wales General (Carmarthen), Ysbyty Glan Clwyd (Rhyl) (including isolates from Maelor Hospital, Wrexham & Ysbyty Gwyndd, Bangor), Royal Glamorgan (Llantrisant), Royal Gwent (Newport) (including isolates from Nevill Hall), Singleton (Swansea) (including isolates from Morriston & Princess of Wales Hospitals), University Hospital of Wales (Cardiff), Withybush (Haverfordwest). Unfortunately only the Royal Gwent and Royal Glamorgan laboratories provided the requested number of isolates to enable trend analysis.

Table 3: Number of isolates received from participating laboratories

Hospital Location	No Req'd	Hospital E. coli	Hospital Coliform	GP E. coli	GP Coliform	Total Rec'd
Royal Gwent, Newport	135	80	21	134	25	260
UHW, Cardiff	147	46	12	72	5	135
Singleton, Swansea	196	8	9	2	1	20
WWGH, Carmarthen	75	14	3	22	6	45
Royal Glamorgan, Llantrisant	41	27	2	41	5	75
Ysbyty Glan Clwyd, Rhyl	230	17	3	14	4	36
Withybush, Haverfordwest	36	7	2	15	3	27
Total	860	199	52	300	49	600

3.2 Sample Type

The majority of isolates submitted originated from urine samples, (>98%), from the community, outpatients and inpatients. The remainder originate from blood cultures.

3.3 Cephalosporin Susceptibility

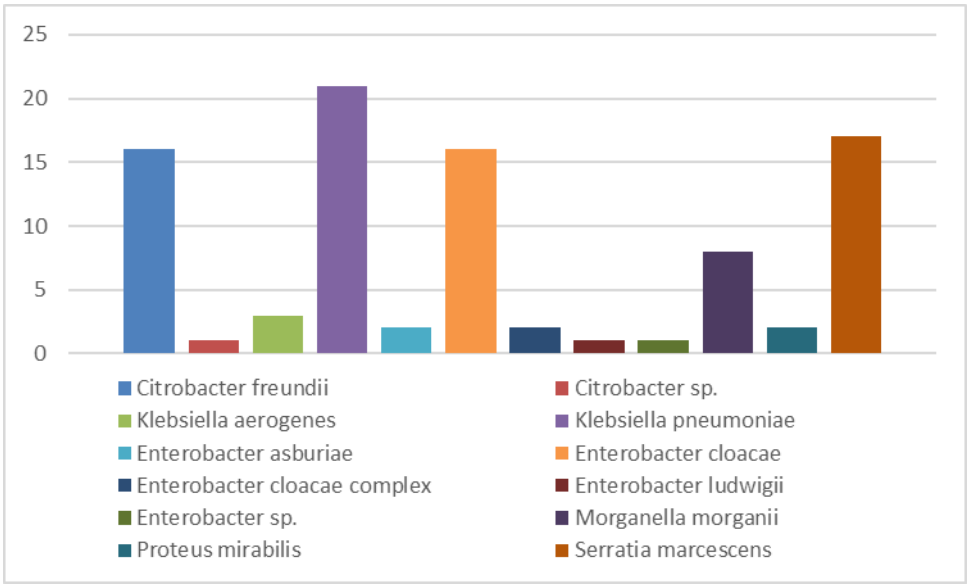
Of the 600 isolates received, 517 (86.2%) isolates were confirmed resistant (R) to 3rd generation cephalosporin (cefotaxime and ceftazidime) by broth microdilution. 49 (8.2%) were categorised as Susceptible, Increased exposure (I) and 34 (5.6%) confirmed as susceptible (S). The 3rd generation cephalosporin susceptible isolates were devoid of any obvious resistance mechanism, such as ESBL or hyper expressed ampC β-lactamase. Of the 49 isolates categorised as Increased exposure, 23 (47%) had either an ESBL or other β-lactamase responsible for the higher cephalosporin MICs. The remaining isolates (53%) contained no non-native resistance mechanism.

In comparison with isolates received in 2008, similar levels of resistance and susceptibility was confirmed. 95.1% of isolates received in 2008 were confirmed either resistant (R) or Intermediate (I) to 3rd generation cephalosporins (compared with 94.5% in 2014), whilst susceptible isolates made up 4.9%. For this report, a change in definition of the "Intermediate" category to "Increased exposure" has led to these isolates being coalesced with the susceptible isolates as per EUCAST guidance.

3.4 Diversity of Species

Of the 517 3rd generation cephalosporin resistant isolates 427 (82.6%) were *Escherichia coli*, 24 (4.6%) were *Klebsiella* spp., 22 (4.2%) were *Enterobacter* spp., 17 (3.3%) were *Citrobacter* spp., 17 (3.3%) were *Serratia* spp., 8 (1.5%) were *Morganella* spp. and 2 (0.4%) were *Proteus* spp. (Figure 1).

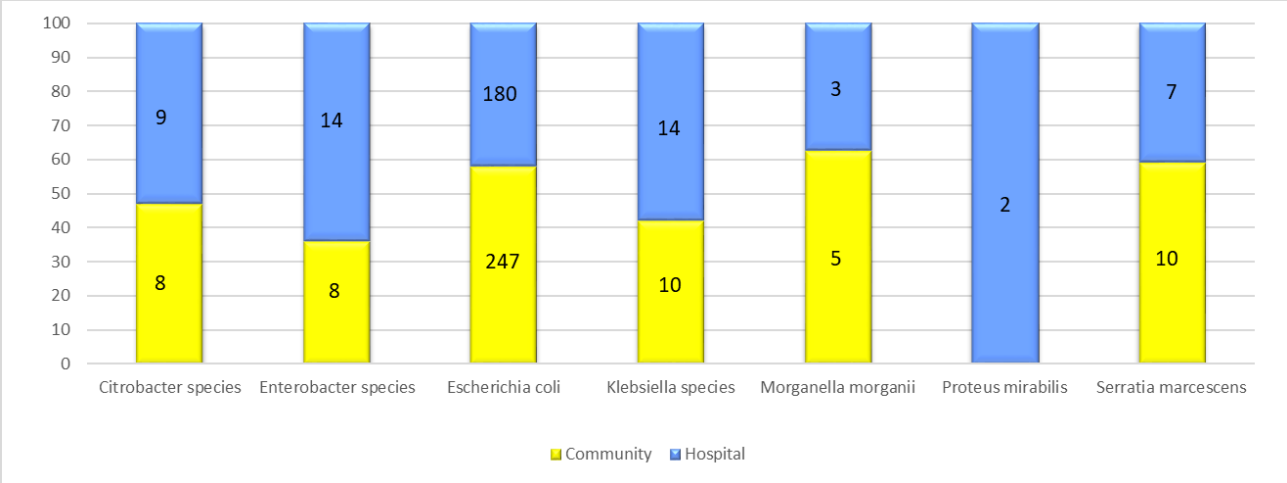
Figure 1: Diversity of non-*E. coli* coliform species received (17.4% of total)



Note: *Enterobacter aerogenes* has recently been reclassified as *Klebsiella aerogenes*.

The proportion of isolates originating from hospital or community setting is shown below. (Figure 2).

Figure 2: Percentage of hospital (H) and community (C) isolates within species (numbers of isolate shown as numbers within the bar).



3.5 Resistance Mechanisms

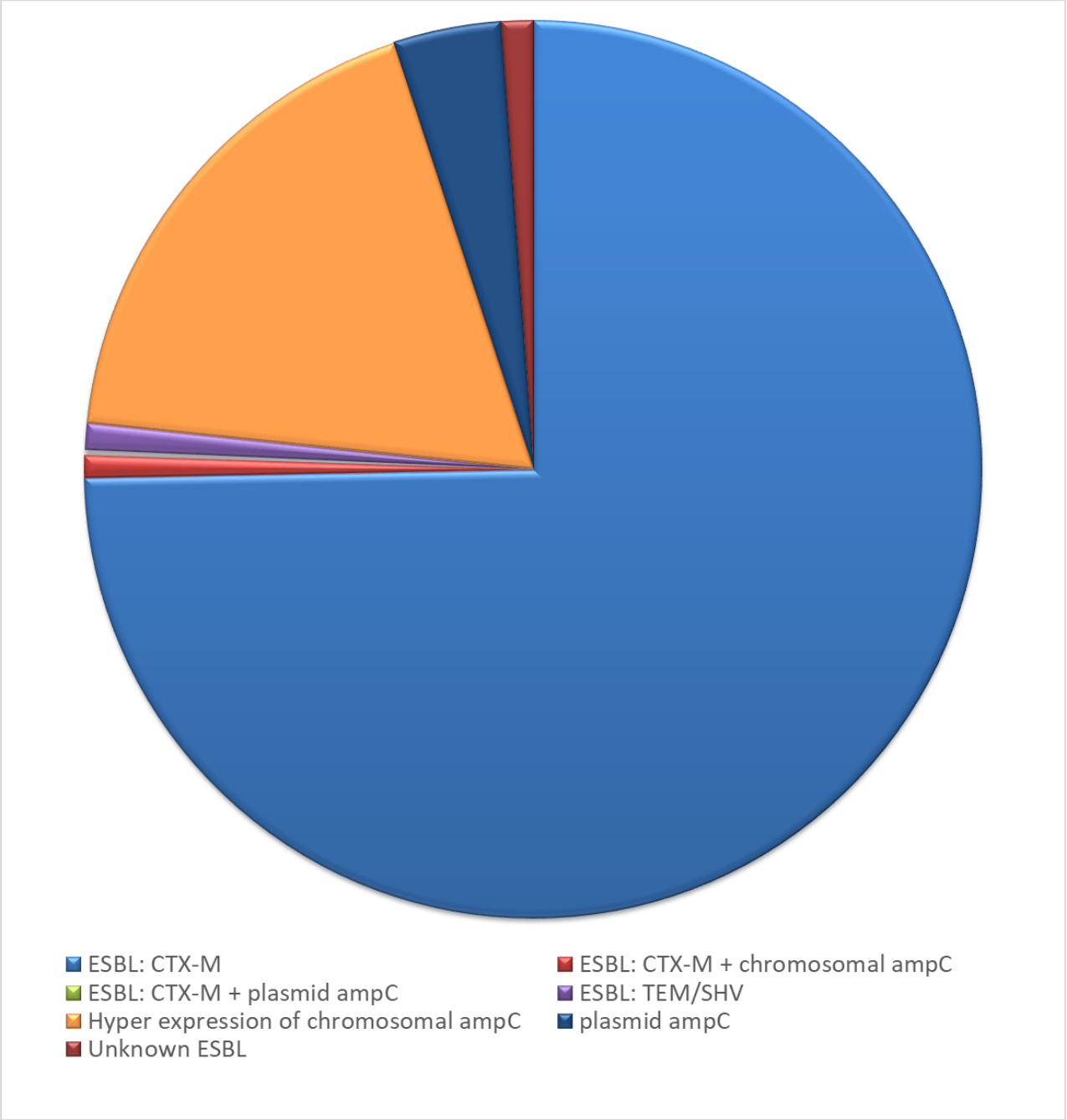
Resistance Mechanisms Conferring 3rd Generation Cephalosporin Resistance in Enterobacterales Isolates Wales-Wide

Of the 517 3rd generation cephalosporin resistant isolates 392 (75.7%) contained a CTX-M ESBL; 368 (71.2%) isolates where CTX-M ESBL was the only resistance mechanism, 23 (4.5%) isolates also exhibiting hyper expression of the native chromosomal ampC, plus 1 (0.2%) isolate also with an acquired plasmid ampC (CIT) (Figure 3 & Table 4).

Table 4: Resistance mechanisms conferring 3rd generation cephalosporin resistant isolates: 2008 & 2014

Resistance mechanism	No (2014)	% (2014)	No (2008)	% (2008)
ESBL: CTX-M	368	71.2%	305	52.6%
ESBL: CTX-M + hyper expression of chromosomal ampC	23	4.5%	37	6.4%
ESBL: CTX-M + plasmid ampC	1	0.2%	4	0.7%
ESBL: TEM/SHV	5	0.97%	32	5.5%
ESBL: TEM/SHV + hyper expression of chromosomal ampC	0	0%	26	4.5%
Hyper expression of chromosomal ampC	94	18.2%	138	13.8%
Plasmid ampC	20	3.9%	16	2.8%
Unknown ESBL	5	1.2%	18	3.1%
Unknown ESBL + hyper expression of chromosomal ampC	1	0.2%	0	0%
K1 (*K. oxytoca*)	0	0%	4	0.5%
Total	517	100%	580	100%

Figure 3: Breakdown of resistance mechanisms in 3rd generation cephalosporin resistant isolates (2014)



The numbers and percentage of Enterobacterales with CTX-M ESBLs in 2014 was 392 (75.7%), an increase in comparison with 346 (59.6%) in 2008. The number of isolates harbouring CTX-M ESBLs alone was 368 (71.2%) in 2014 compared with 305 (52.6%) in 2008. The number of isolates with CTX-M in combination with either hyper expression of ampC or plasmid ampC was lower in 2014 (c-ampC:23, p-ampC:1) compared with in 2008 (c-ampC:37, p-ampC:4).

In 2014, 11 (2.1%) isolates contained ESBLs other than CTX-M, 1 (0.2%) harboured TEM, 3 (0.6%) harboured SHV and 1 (0.2%) harboured SHV and TEM ESBLs. No isolates contained a TEM or SHV ESBL and hyper-expression of a native chromosomal ampC however, 6 (1.2%)

isolates had an unknown ESBL, 1 of which also exhibited hyper-expression of the native ampC. In 2008, the numbers of isolates harbouring non-CTX-M ESBLs, both with and without ampCs were significantly higher (76, 13.1%).

The 2014 figures are in line with the known upward trend in CTX-M ESBLs replacing TEM/SHV ESBLs in Enterobacterales. A similar UK-based study which reports 71.6% of 3rd generation cephalosporin resistant *Enterobacterales* containing CTX-M ESBLs and ≤16% harbouring non-CTX-M ESBLs and oxacillinases (Reid et al Am J of BioMed Science & Res 2019). For 2008 data, contemporary studies site similar lower rates of CTX-M containing isolates (44.7%) in 3rd generation cephalosporin resistant *Enterobacterales,* with 13.3% containing non-CTX-M ESBLs and 16.9% with hyper expressed ampC β-lactamases.[3]

The number of isolates where 3rd generation cephalosporin resistance is conferred by hyper expression of the native ampC or acquisition of plasmid ampC β-lactamases alone were 94 (18.2%) and 20 (3.9%) in 2014 compared with 138 (13.8%) and 16 (2.8%) in 2008. A slight increase in both was found in 2014.

Overall, 20 isolates contained plasmid-mediated ampC ß-lactamases, of which 19 were the sole mechanism of resistance to 3rd generation cephalosporins, in 1 isolate it was combined with a CTX-M group 9 CTX-M. Plasmid-mediated ampC ß-lactamases were seen equally in isolates from the community and hospital, with 19 conferring resistance in *E. coli* and 1 in *P. mirabilis*.

The majority (18, 90%) of the plasmid-mediated ampC ß-lactamases found in Welsh isolates were CIT type. CIT type are the most commonly acquired plasmid ampC ß-lactamases enzyme in the UK, followed by ACC, FOX and DHA types. CIT ampC genes originated and escaped from the *Citrobacter freundii*.[6] The other 2 (10%) isolates with plasmid ampC ß-lactamases contained DHA types, from different hospitals (UHW & WWGH); this type originated from *M. morgannii*.

Single and Multiple Resistance Mechanisms

In 491 (94.9%) of the 517 isolates 3rd generation cephalosporin resistance was conferred by a single resistance mechanism. 3rd generation cephalosporin resistance was caused by multiple resistance mechanisms in 26 (5.1%) isolates (Table 4).

Species Specific Resistance Mechanisms

CTX-M genes were found predominantly in *E. coli* and *K. pneumoniae* (Table 5 & Figure 4), accounting for 80% and 100% of 3rd generation cephalosporin resistance respectively.

3rd generation cephalosporin resistance in *Enterobacter* species, *K. aerogenes*, *Citrobacter* species, *Morganella morgannii*, *Proteus mirabilis* and *Serratia* marcescens was mainly conferred by hyper production of chromosomal β-lactamases. A significant number of isolates with hyper expressed native chromosomal ampC were E. coli. E. coli ampCs generally confer resistance to ampicillin/amoxicillin only. However, acquisition of a new promoter causes the native chromosomal ampC enzyme to hyper express, causing resistance to 3rd generation cephalosporins. AmpC β-lactamase enzymes were sometimes found to contribute to 3rd generation cephalosporin resistance in combination with an ESBL, predominantly in *E. coli*.

Plasmid mediated ampC β-lactamases were found mainly in *E. coli* (20, 3.9%) and in 1 *P. mirabilis* isolate. It is understood that plasmids known to carry CTX-M or other ESBLs can also harbour other resistance genes such as ampC β-lactamases. A plasmid mediated ampC β-lactamase is in combination with a CTX ESBL in an *E. coli* isolate and an unknown ESBL in an *Enterobacter* species (Table 5).

Figure 4: Resistance mechanisms per species (percentage)

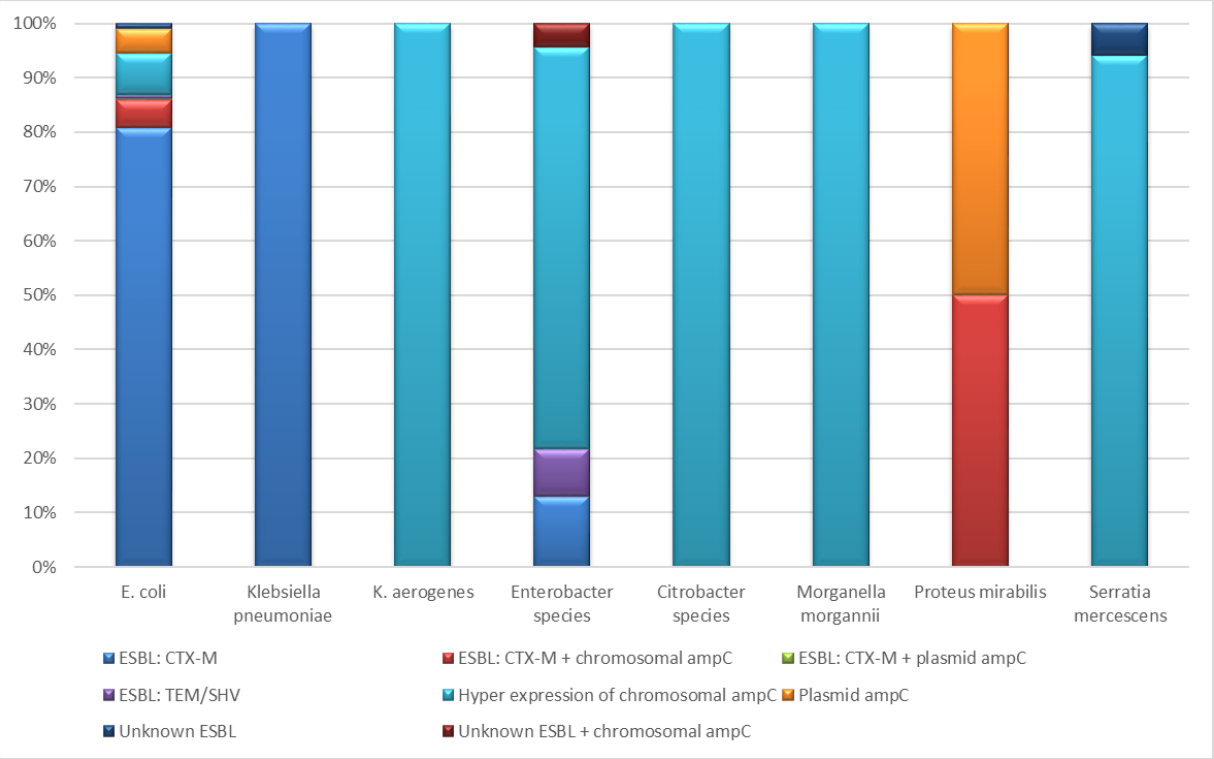


Table 5: Resistance mechanisms per species

Resistance mechanism	ESBL: CTX-M	ESBL:CTX-M + hyper expression of chromosomal ampC	ESBL:CTX-M + hyper expression of plasmid ampC	ESBL: TEM/SHV	Hyper expression of chromosomal ampC	Plasmid ampC	Unknown ESBL	Unknown ESBL + hyper expression of ampC
E. coli	344	22	1	3	33	19	4	
Klebsiella pneumoniae	21							
K. aerogenes					3			
Enterobacter species	3			2	17			1
Citrobacter species					17			
Morganella morgannii					8			
Proteus mirabilis		1				1		
Serratia marcescens					16			

A comparison between 2014 and 2008 suggests that CTX-M ESBLs were more common in *E. coli* (80.7%) in 2014 compared with 2008 (65.8%). The number of isolates where hyper-expression of ampC β-lactamases alone caused 3rd generation cephalosporin resistance in *E. coli* was lower in 2014 (7.7%) compared with 2008 (16.4%).

In 2014, plasmid ampC β-lactamases were mainly found in *E. coli* (20) but 1 was also detected in a *P. mirabilis* isolate. In 2008, plasmid ampC β-lactamases were similarly found in *E. coli* (15) but also a significant number in *K. pneumoniae* (6).

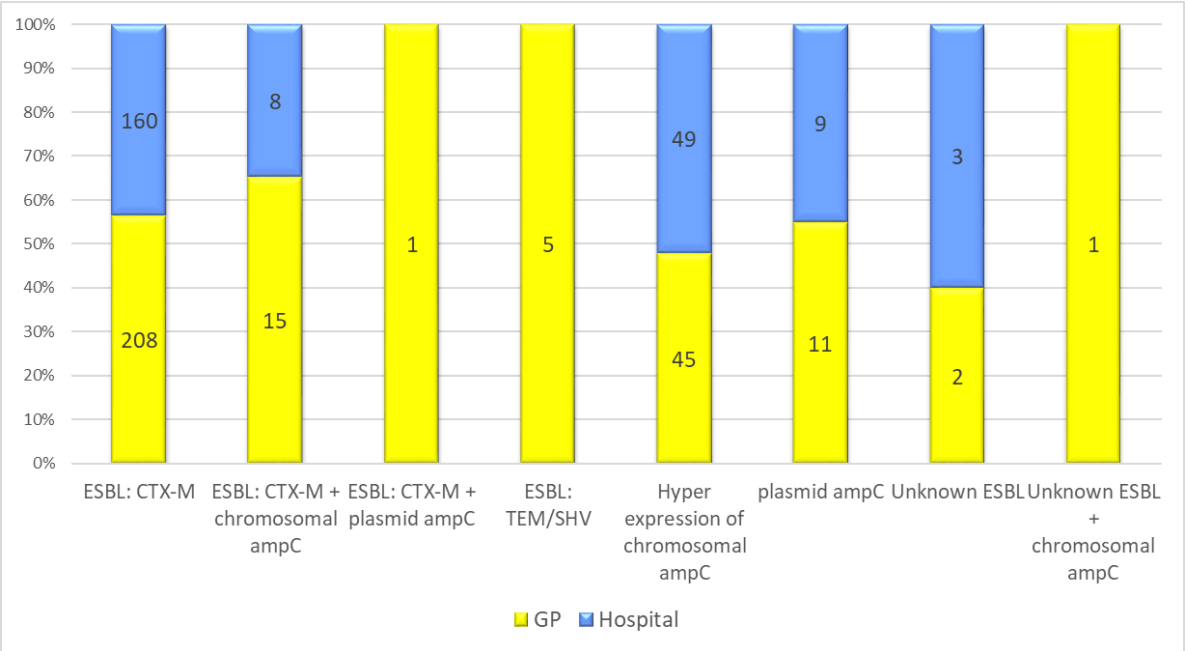
Resistance Mechanisms Predominant in Hospital and Community

ESBLs (CTX-M, TEM, SHV), either alone or in combination with ampC β-lactamases, were more commonly found in isolates from the community than in hospitals; 57% of isolates with CTX-M ESBLs originated in the community compared with 43% from hospital. A similar ratio was seen in 2008.

All 4 isolates which contained a SHV ESBL, 1 in combination with a TEM ESBL, originated from GP samples.

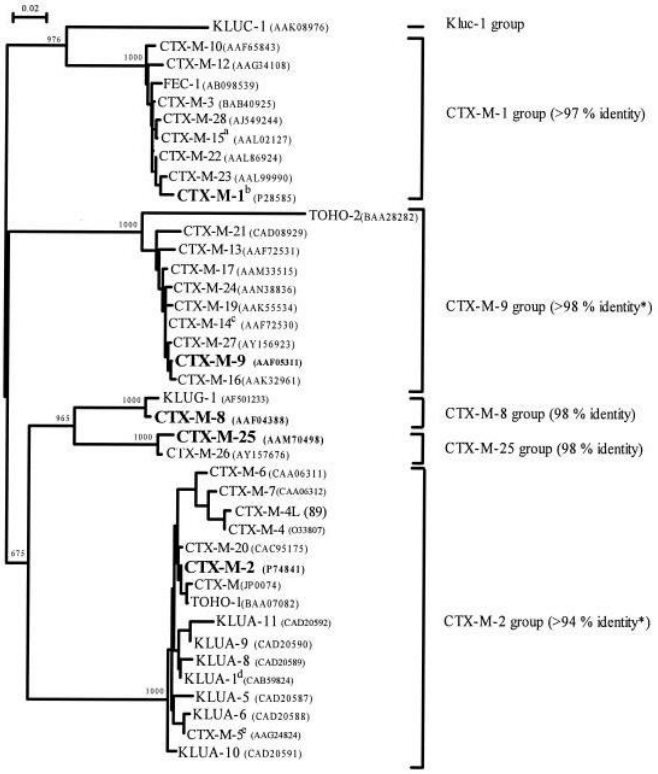
Hyper expression of the native chromosomal ampC or plasmid-mediated ampC β-lactamases were equally found in hospital and GP isolates in 2014. However in 2008, plasmid ampC β-lactamases were more prevalent in the community (GP); 15 (71.4%) compared with 6 (28.6%) in hospital isolates (Figure 5).

Figure 5: Resistance mechanisms prevalent in hospital and community isolates



Diversity of CTX-M Type Groups

There are currently 233 CTX-M type ß-lactamase alleles (variants), however some alleles are more common in the UK than others: CTX-M 15, -14 and -9. For ease of detection by PCR, these alleles are grouped: see below.

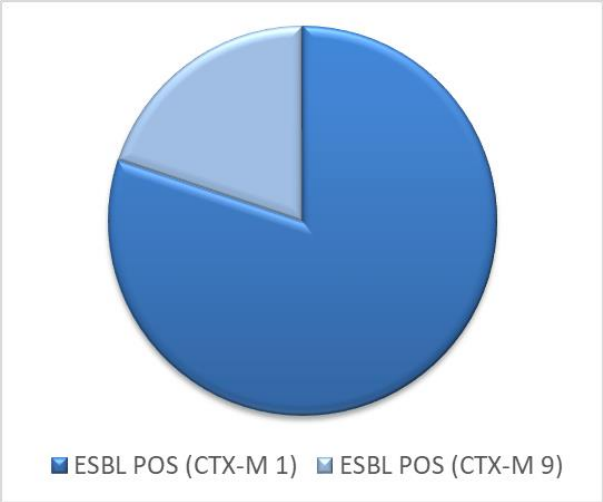


Of the 392 isolates which contained CTX-M genes, 314 (80.1%) were CTX-M group 1 (probably CTX-M-15) and 78 (19.9%) were group 9 (could be CTX-M-9 or CTX-M-14)(Figure 6). No isolates were found to have any other CTX-M group enzymes (groups 2, 8 or 25). In 2008, the predominant CTX-M type was group 1 (CTX-M-15), with the next most abundant being group 9, but found were small numbers containing CTX-M genes from groups 2 and 8.

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 in 90.5% (2008) / 80.1% (2014) is similar to that found in UK data, where 86.4% were CTX-M group 1 genes.

CTX-M group 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. The prevalence of CTX-M group 9 CTX-M in Wales (14.3%) is slightly higher to that in the UK (12.8%); a lower number were seen in Wales in 2008 (7.3%). CTX-M-14 ESBLs have previously been linked with farm animals in Wales.[5] Of the 78 isolates containing CTX-M group 9 ESBLs, 51 (65.4%) originated from the community and 27 (34.6%) originated from hospitals.

Figure 6: Prevalent CTX-M gene groups in Wales



3.6 Resistance Mechanisms Prevalent per Laboratory

The prevalence of isolates with 3rd generation resistance mechanisms are shown below with an All Wales national guide included (Table 6 & Figure 7). Comparisons of resistance mechanisms were only applied to UHW (Cardiff), Royal Gwent (Newport) & Royal Glamorgan (Llantrisant) due to sufficient numbers of isolates submitted.

Figure 7: Breakdown of resistance mechanisms per laboratory (percentage)

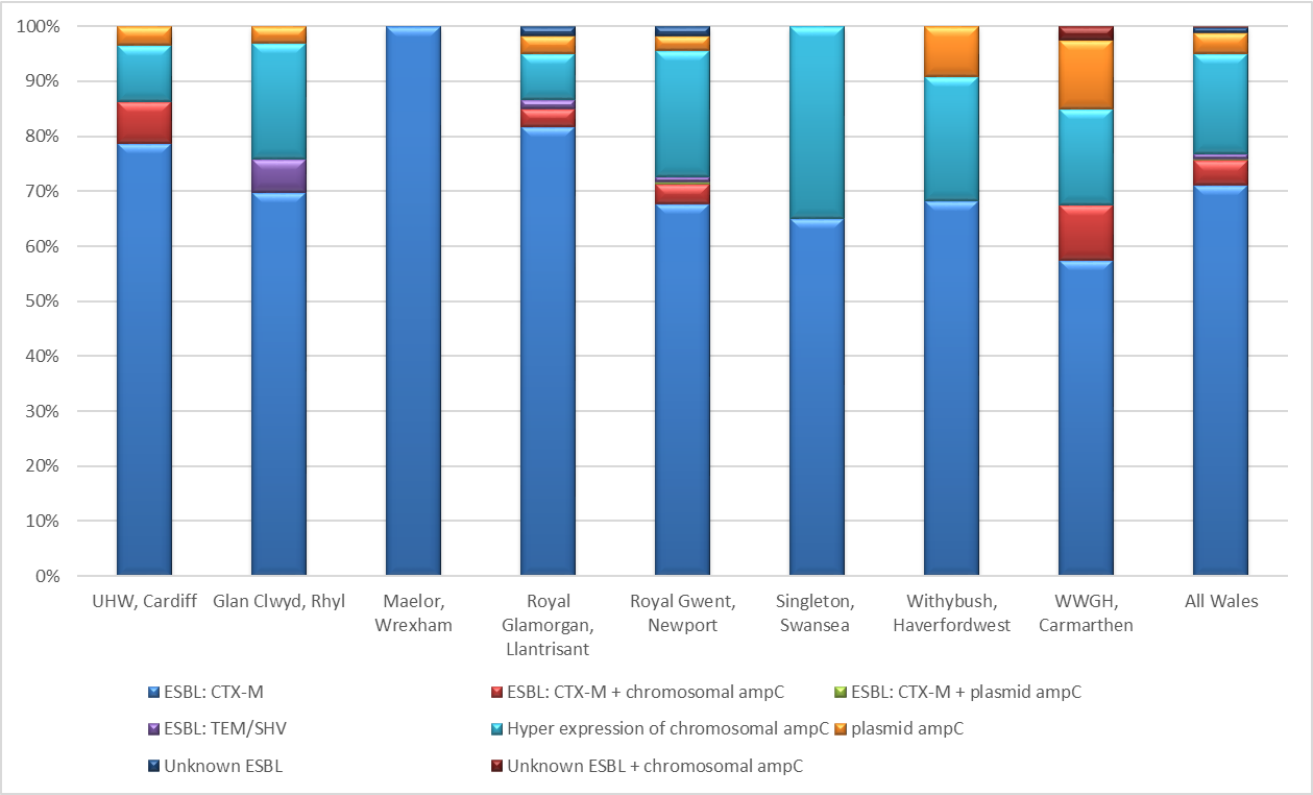


Table 6: Breakdown of resistance mechanisms per laboratory (numbers of isolates)

Resistance mechanism	UHW, Cardiff	Glan Clwyd, Rhyl	Maelor, Wrexham	Royal Glamorgan, Llantrisant	Royal Gwent, Newport	Singleton, Swansea	Withybush, Haverfordwest	WWGH, Camarthen	All Wales
ESBL: CTX-M	92	23	2	49	151	13	15	23	368
ESBL: CTX-M + chromosomal ampC	9			2	8			4	23
ESBL: CTX-M + plasmid ampC					1				1
ESBL: TEM/SHV		2		1	2				5
Hyper expression of chromosomal ampC	12	7		5	51	7	5	7	94
Plasmid ampC	4	1		2	6		2	5	20
Unknown ESBL				1	4				5
Unknown ESBL + chromosomal ampC								1	1

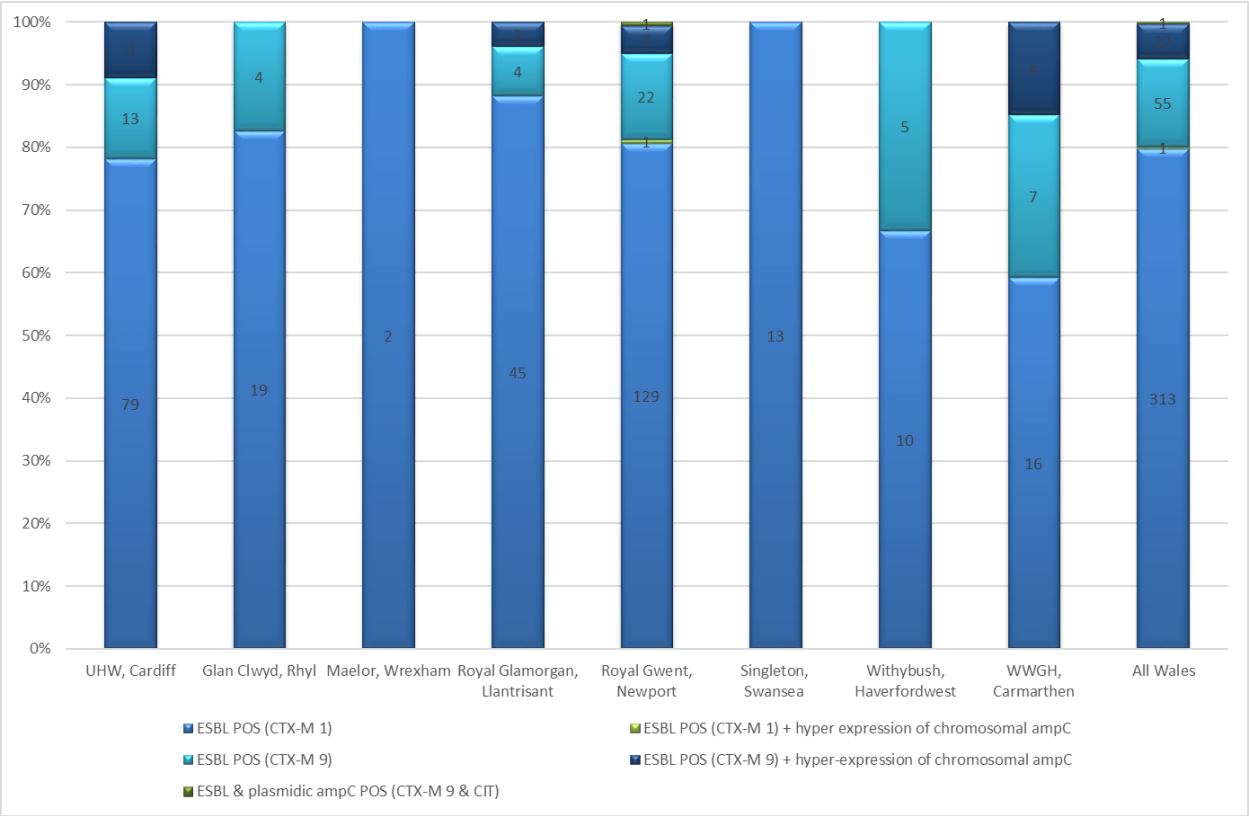
CTX-M type ß-lactamases

The percentage of Enterobacterales from All Wales where CTX-M genes confer resistance to 3rd generation cephalosporins is 71.2%. The percentage of isolates harbouring CTX-M ESBLs ranged from 57.5% in WWGH (Carmarthen) to 81.7% in the Royal Glamorgan hospital (Llantrisant) (Figure 8).

The majority of isolates harbouring CTX-M ESBLs did not contain other 3rd generation cephalosporin resistance mechanisms. Of note is the *E. coli* from the Royal Gwent hospital (Newport) which contained a CTX-M-9 gene plus a plasmid-mediated ampC (CIT).

With regard to the diversity of CTX-M alleles (variants), the majority of CTX-M ESBLs in all laboratories was group 1 (probably CTX-M-15), the most common variant found in the UK. This was followed by group 9 (probably CTX-M-14 or CTX-M-9), the second and third most common in the UK. However, the percentage of CTX-M group 9 ESBLs in isolates from Withybush and WWGH were higher, similar to levels found in 2008. This suggests a successful lineage of CTX-M group 9 ESBLs in these geographical areas, potentially with a link to farming.[4,5]

Figure 8: CTX-M ESBL variants per laboratory (percentage (graph) & numbers)



SHV & TEM type ESBL ß-lactamases

The 5 isolates containing TEM and/or SHV ESBLs were submitted from 3 hospital laboratories: Royal Gwent (Newport), Royal Glamorgan (Llantrisant) and Glan Clwyd (Rhyl). The 2 isolates from Glan Clwyd were both E. cloacae isolates, 1 harbouring a SHV whilst the other contained both TEM and SHV ESBLs.

AmpC ß-lactamases

Hyper- expressed chromosomal ampC ß-lactamases

Most laboratories had similar percentages of isolates where hyper-expression of the chromosomal ampC β-lactamase was the sole 3rd generation cephalosporin resistance mechanism (Figure 7 & Table 6). The range of percentages of isolates where hyper-expressed ampC β-lactamase was the sole mechanism of resistance ranged from 10.3% in UHW (Cardiff) to 22.9% in the Royal Gwent Hospital (Newport).

Plasmid-mediated ampC ß-lactamases

Isolates containing plasmid-mediated ampC ß-lactamases were found in UHW (Cardiff), Glan Clwyd (Rhyl), Royal Glamorgan (Llantrisant), Royal Gwent (Newport), Withbush (Haverfordwest) and WWGH (Carmarthen) laboratories but not in Maelor (Wrexham) or Singleton (Swansea). In most cases, the plasmid-mediated ampC ß-lactamase was present alone (Figure 7 & Table 6).

The majority of plasmid ampC ß-lactamases were CIT (18, 90%) plus 2 (10%) isolates with with DHA types, the latter from different hospitals (UHW & WWGH).

3.7 Resistance Mechanisms in Isolates Categorised as Susceptible, increased exposure (I) to 3rd Generation Cephalosporins.

49 (8.2%) isolates were categorised as S, increased exposure (I) for a 3rd generation cephalosporin. Over half (25, 51%) of these isolates contained no discernible resistance mechanism which confer resistance to 3rd generation cephalosporins. Hyper expression of the native chromosomal ampC β-lactamases were present in 17 (34.7%) isolates, the majority (15) were *E. coli* but 2 were *S. marcescens*. In these isolates the mean ceftazidime MIC was 4mg/L, very near the clinical breakpoint for resistance (Table 7).

A plasmid ampC gene (DHA) was detected in 1 *E. coli* isolate from UHW, Cardiff, with a ceftazidime MIC of 4mg/L, near the clinical breakpoint for resistance.

Overall, the EUCAST 3rd generation cephalosporin clinical breakpoints performed well at dividing susceptible from resistant isolates.

Table 7: Resistance mechanisms found in isolates categorised as Susceptible, increased exposure (I)

Resistance mechanism	No	%
None detected	26	53.1%
ESBL: CTX-M group 1	1	2%
Hyper expression of chromosomal ampC	17	34.7%
Plasmid ampC (DHA)	1	2%

3.8 Correlation of Cephalosporin MICs with Resistance Mechanisms

Antibiograms, the resistance profile of any isolate, can sometimes reveal the underlying resistance mechanism. The following sections show how susceptibility/resistance to certain 3rd generation cephalosporins can be used to predict the mechanism of resistance.

Table 8: Differential mean 3rd generation cephalosporin MICs per resistance mechanism

Resistance mechanism	CTX MIC Range (mg/L)	CTX GeoMEAN (mg/L)	CAZ MIC Range (mg/L)	CAZ GeoMEAN (mg/L)
ESBL: CTX-M	4->128	102.7	0.12->128	19.2
ESBL: NON-CTX-M (TEM/SHV/Unknown)	0.25-64	2.8	16->128	29.6
Hyper expression of chromosomal ampC	0.5->128	11.1	0.5->128	12.6
Plasmid ampC	2-64	10.2	8->128	29.7

CTX: cefotaxime, CAZ: ceftazidime

ESBL ß-lactamases

It is well established that CTX-M enzymes hydrolyse cefotaxime preferentially over ceftazidime, leading to higher cefotaxime MICs in isolates containing CTX-M enzymes. The mean cefotaxime MIC for isolates containing a CTX-M gene is 102.7mg/L, compared with 2.8mg/L for isolates with other ESBLs (Table 8).

In comparison, mean ceftazidime MICs were generally higher in non CTX-M ESBLs (29.6mg/L) than for isolates harbouring CTX-M ESBLs (19.2mg/L).

AmpC ß-lactamases

AmpC β-lactamases do not preferentially hydrolyse cefotaxime of cetazidime. The mean cefotaxime and ceftazidime MICs for isolates with hyper-expressing chromosomal ampCs are similar; 11.1mg/L & 12.6mg/L respectively. However in isolates with plasmid ampC β-lactamases, the mean ceftazidime MIC was significantly higher (29.7mg/L).

AmpC β-lactamases do preferentially hydrolyse cefoxitin. This agent is rarely tested for clinical purposes but is useful for identification of ampC resistance mechanisms. Cefoxitin mean MICs for isolates with either a hyper expressed chromosomal ampC or plasmid ampC β-lactamase is significantly higher than for isolates containing ESBLs (Table 9). Further the mean cefoxtin MIC is higher for isolates containing plasmid ampCs (60.7mg/L) than for those with hyper expressed native chromosomal ampCs (50.6mg/L). This confirms that cefoxitin resistance is a good indicator for presence of ampC β-lactamases.

Table 9: Differential mean cefoxitin MICs per resistance mechanism

Resistance mechanism	FOX MIC Range (mg/L)	FOX GeoMEAN (mg/L)
ESBL: CTX-M	0.12->128	8.2
ESBL: NON-CTX-M (TEM/SHV/Unknown)	4->128	20.7
Hyper expression of chromosomal ampC	8->128	50.6
Plasmid ampC	8->128	60.7

FOX: cefoxitin

3.9 Resistance to other agents

Ciprofloxacin

Resistance mechanisms for ciprofloxacin and 3rd generation cephalosporin resistance are not associated directly. This is evident in the relatively low percentage ciprofloxacin resistance in isolates with either traditionally chromosomal ESBLs (TEM/SHV) and hyper expression of native chromosomal ampCs (Table 10). However isolates with CTX-M ESBLs (mostly carried on plasmids) and plasmid-mediated ampCs exhibited higher ciprofloxacin MICs (79.6% and 80% resistance).

Table 10: Ciprofloxacin susceptibility per resistance mechanism

Resistance mechanism	CIP MIC Range (mg/L)	CIP GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	0.008->128	7.5	20.4%/79.6%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	0.008-64	0.4	60%/40%
Hyper expression of chromosomal ampC	0.008->128	0.1	76.6%/23.4%
Plasmid ampC	2-64	0.1	20%/80%

CIP: ciprofloxacin

Gentamicin

Resistance mechanisms for gentamicin and 3rd generation cephalosporin resistance are not associated directly. However, as for ciprofloxacin, the acquisition of plasmid mediated ampC β-lactamase seems to be associated with higher (90%) gentamicin resistance (Table 11).

Table 11: Gentamicin susceptibility per resistance mechanism

Resistance mechanism	GEN MIC Range (mg/L)	GEN GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	0.25->128	4.4	58.4%/41.6%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	0.5->128	4	40%/60%
Hyper expression of chromosomal ampC	0.12-128	0.8	92.6%/7.4%
Plasmid ampC	0.5-128	2.7	10%/90%

GEN: gentamicin

Mecillinam

Resistance mechanisms for mecillinam and 3rd generation cephalosporin resistance are not associated directly. Unlike most β –lactam agents, which bind to Gram-negative PBP-1A, -1B or -3, mecillinam binds to PBP2. Mecillinam has shown excellent activity against isolates with a range of resistance mechanisms.[7] Low resistance rates were seen in most isolates, irrespective of 3rd generation cephalosporin resistance mechanism (Table 12).

Table 12: Mecillinam susceptibility per resistance mechanism

Resistance mechanism	MEC MIC Range (mg/L)	MEC GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	0.06->128	1.2	94.6%/5.4%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	0.25->128	3.2	80%/20%
Hyper expression of chromosomal ampC	0.06->128	0.8	67%/33%
Plasmid ampC	0.25->128	1.3	85%/15%

MEC: mecillinam

Nitrofurantoin

Resistance mechanisms for nitrofurantoin and 3rd generation cephalosporin resistance are not associated directly. Low levels of resistance to nitrofurantoin were seen in isolates, irrespective of resistance mechanism (Table 13).

Table 13: Nitrofurantoin susceptibility per resistance mechanism

Resistance mechanism	NIT MIC Range (mg/L)	NIT GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	1->128	19	86.1%/13.9%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	4->128	17.4	70%/30%
Hyper expression of chromosomal ampC	1->128	25.7	74.5%/25.5%
Plasmid ampC	0.12->128	16	90%/10%

NIT: nitrofurantoin

Temocillin

Resistance mechanisms for temocillin and 3rd generation cephalosporin resistance are not associated directly. Temocillin is a derivative of ticarcillin and has shown good activity against Gram-negatives with both ESBLs and ampC β-lactamases.[8] Welsh data suggests that isolates with a CTX-M ESBL in particular have low temocillin MICs (84.5% susceptible) (Table 14).

Table 14: Temocillin susceptibility per resistance mechanism

Resistance mechanism	TEM MIC Range (mg/L)	TEM GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	1->128	6.2	84.5%/15.5%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	2-32	7.5	70%/30%
Hyper expression of chromosomal ampC	1->128	8	67%/33%
Plasmid ampC	2-32	6.5	90%/10%

TEM: temocillin

Meropenem

Presence of ESBLs and ampC β-lactamases in Enterobacterales can confer low level resistance to meropenem in some Gram-negative isolates. All isolates, irrespective of cephalosporin resistance mechanism, were susceptible to meropenem. (Table 15).

Table 15: Meropenem susceptibility per resistance mechanism

Resistance mechanism	MER MIC Range (mg/L)	MER GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	0.008-0.5	0.03	100%/0%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	0.015-0.12	0.03	100%/0%
Hyper expression of chromosomal ampC	0.015-8	0.05	100%/0%
Plasmid ampC	0.015-0.06	0.03	100%/0%

MER: meropenem

Colistin

Resistance mechanisms for colistin and 3rd generation cephalosporin resistance are not associated directly. The majority of isolates, irrespective of cephalosporin resistance mechanism, were susceptible to colistin (Table 16). Those isolates testing resistant to colistin were predominantly *Serratia*, *Morganella* and *Proteus* species, known to be inherently resistant to colistin (MICs 128mg/L). There were 10 other colistin resistant isolates, 6 *E. coli*, 3 *Enterobacter* species and 1 *C. freundii*, which had colistin MICs 4->128mg/L. No isolates contained the plasmid mediated mobile colistin resistance (mcr) genes.

Table 16: Colistin susceptibility per resistance mechanism

Resistance mechanism	COL MIC Range (mg/L)	COL GeoMEAN (mg/L)	% S / % R
ESBL: CTX-M	0.06-128	0.2	98.9%/1.1%
ESBL: NON-CTX-M (TEM/SHV/Unknown)	0.06->128	0.1	90%/10%
Hyper expression of chromosomal ampC	0.03->128	0.2	71.2%/28.7%
Plasmid ampC	0.12->128	0.2	95%/5%

COL: colistin

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