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Reduced penicillin susceptibility of group B Streptococcus: an assessment of emergence in Grampian, Scotland

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Group B Streptococcus (GBS; *Streptococcus agalactiae*) can be found as a part of the normal intestinal and vaginal flora in healthy adults.[1] However, they are also the leading cause of neonatal sepsis and meningitis.[2] In addition, invasive GBS diseases are increasingly being reported in non-pregnant adults,[3,4] particularly in the elderly and those with comorbidities such as diabetes.[5,6]

Penicillins are the first line agents for prophylaxis and the treatment of these GBS infections.[7] Since clinically isolated GBS are considered to be uniformly susceptible to β-lactams[8], limited susceptibility testing is routinely performed. Nevertheless, GBS isolates with reduced penicillin susceptibility (PRGBS) have been identified periodically in Japan, North America and elsewhere on occasion at frequencies of 1/10 to 1/200.[9–14] These PRGBS isolates were found to have penicillin minimum inhibitory concentrations (MICs) of 0.25–1.0 mg/L, values that lie above the susceptibility breakpoint for penicillin of 0.12 mg/L, determined by the Clinical and Laboratory Standards Institute (CLSI).[8] In 2009, Kimura and colleagues reported that susceptibility testing of their PRGBS isolates by the CLSI standard disc diffusion method using only a penicillin disc failed to discriminate between these and GBS isolates sensitive to penicillin.[15] Consequently, they developed a more detailed screening method aimed at detecting clinical cases of PRGBS in an easy and reliable manner.

To date, there are no reports of PRGBS in the UK.[16,17] Accordingly, we set out to characterise the resistance patterns of GBS isolates present in the patient population of the Grampian region in Scotland, UK. The reasoning for this was twofold: firstly to assess any emergence of PRGBS isolates in our patient population; secondly, to establish whether sensitivity analysis should be implemented for all β-haemolytic Streptococci clinical isolates, which are currently reported automatically as sensitive to penicillin.

Two hundred GBS isolates were collected from consecutive genital specimens received by Aberdeen Royal Infirmary between February 2010 and April 2010 to represent the general bacterial population in the region and eliminate selection bias. The GBS isolates were identified by their colony morphology, Gram reaction and appearance on chromID® Strepto B agar (bioMérieux, Marcy l'Etoile, France).[18] Those that gave a positive reaction on the agar were tested for antimicrobial susceptibility.

Susceptibility testing for PRGBS was performed following the screening method developed by Kimura et al.[15] This method recommends susceptibility testing with four discs as the penicillin disc alone frequently gave growth-inhibitory zones >24 mm (CLSI susceptibility criteria) despite the isolate showing signs of reduced susceptibility. Penicillin (10 U), oxacillin (1 µg), ceftizoxime (30 µg) and ceftibuten (30 µg) (Oxoid, Hants, UK) discs were used, following the CLSI guidelines for disc diffusion testing of beta-haemolytic streptococci.[8] Zone sizes were measured using callipers and compared with published breakpoints for which the CLSI had not determined the cut-off values for susceptible criteria.[15] For isolates that showed reduced susceptibility to two of the discs – suggestive of reduced penicillin susceptibility according to the method by Kimura et al.[15] – the penicillin MIC for the isolate was measured using an Etest® strip, according to the manufacturer's protocol (bioMérieux, Inc., Durham, NC, USA).

All isolates had an inhibitory zone size of >24 mm to penicillin suggesting no reduced susceptibility. A small minority of isolates displayed resistance to at least one of the other antibiotics tested (Table 1). 2.5% of isolates had reduced susceptibility to two antibiotics and 19.5% had reduced susceptibility to one antibiotic.

Sensitivity testing using Etest strips was performed on five of the isolates according to the above criteria, none of which displayed reduced susceptibility to penicillin – MICs ranged from 0.064 to 0.094 mg/L – despite showing reduced susceptibility to two of the discs (Table 2).

Our data suggest that GBS isolates with reduced penicillin susceptibility are not emerging in the patient population of the Grampian area in Scotland, UK. Of the two hundred isolates tested, none were found to have a reduced zone to penicillin or – when tested – an increased MIC.

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Table 1. Antimicrobial susceptibility and range of growth-inhibitory zone diameters in group B Streptococcus isolates.

	Susceptibility		Growth-inhibitory zone diameter (mm)		
Antimicrobial	*S* (%)	*R* (%)	Susceptible[a]	Mean	Variance
Penicillin	100	0	≥24	32.53	3.17
Oxacillin	99.0	1.0	≥17	19.67	1.79
Ceftizoxime	98.5	1.5	≥29	32.39	5.48
Ceftibuten	78.0	22.0	≥20	21.03	2.02

S is sensitive; R is resistant.
[a]Criteria set by Kimura et al.[15]

Table 2. MICs obtained by Etest for five GBS isolates that displayed growth within the growth-inhibitory zones around two of oxacillin, ceftizoxime and ceftibuten discs.

		Diameter (mm) inhibitory-zone with:		
Isolate No.	MIC (mg/L)	Oxacillin	Ceftizoxime	Ceftibuten
19	0.094	**16**	32	**17**
20	0.094	18	**27**	**19**
39	0.064	18	**9**	**19**
50	0.094	18	**28**	**19**
94	0.094	**16**	29	**19**

Numbers in bold identify where the growth was within the inhibitory zone.
MIC = minimum inhibitory concentration.

PRGBS have been identified elsewhere in a number of different studies, using various methodologies and emerging at varying frequencies. Kimura and colleagues characterised fourteen PRGBS isolates; nine of these were collected from 1995 to 1998 and five were obtained from a screen of all one hundred and fifty-nine samples received over a three-day period in 2005.[9] The isolates were from the sputa of individual patients, most of whom were elderly and none were from sterile sites e.g. the blood, cerebrospinal fluid (CSF). Conversely, Murayama et al. only probed isolates from sterile sites including the blood, CSF pustule fluid, joint fluid and tissue from children and adults.[11] They investigated all one hundred and eighty-nine isolates sent to them between August 2006 and July 2007 and found that one had mutations in the *pbp2x* gene associated with reduced penicillin susceptibility. A further study in Japan identified no PRGBS isolates from two hundred, and then extended to two thousand consecutively collected vaginal/rectal isolates.[19] Two papers reported individual cases of PRGBS isolates from the surgical site following a hip replacement.[13,14] In both cases, the GBS infection initially showed sensitivity to penicillin, but recurrence three and six years later showed raised MICs, above the susceptibility breakpoint of 0.12 mg/L defined by the CLSI.[8] Studies in neonates have also yielded differing results; forty isolates from infected neonates were tested for PRGBS in China, none of which had raised MICs over 0.12 mg/L.[20] A Swedish study of one hundred and seventy-four adults and one hundred and twenty-three neonates found two isolates with MICs of 0.25 mg/L.[12]

The differing results from these studies, including ours, highlight the variability surrounding the emergence of PRGBS isolates. Whilst penicillin may still remain the drug of choice for treating GBS infections, arguments have been made for performance of sensitivity analysis against GBS to become routine.[12] Currently, the UK defines GBS as penicillin sensitive.[16,17] If PRGBS isolates do begin to emerge in the patient population, an easy and reliable screening method is required, since penicillin discs alone have been found to be unreliable at differentiating between penicillin-sensitive GBS strains and PRGBS.[15]

Kasahara et al.[19] reported that the proposed breakpoints and screening tests described by Kimura et al.[15] had "an unacceptably high false-positive rate". The methodology describe in Kasahara et al.[19] shares most similarities with this current study including the same initial sample size and the consecutive collection of isolates from vaginal/rectal samples. Our data support their observation, despite the five isolates indicating reduced penicillin sensitivity according to the criteria set out by Kimura et al.[15] Using Etest strips, they produced MIC values of 0.064–0.094 mg/L, which were within the sensitivity range (≤0.12 mg/L) according to the CLSI.[8] These data indicate that the specificity of the proposed method is too low, leading to a significant number of false-positive results. For this reason, we are unable to advocate its use as a screening method for PRGBS. For those countries where PRGBS is already a concern and in case of the emergence of PRGBS in the UK, further efforts must be undertaken to develop a more robust screening method. This is particularly important if measuring the MIC by Etest alone is deemed too costly.

This report represents an advance in biomedical science because it highlights the necessity for the development of a more robust screening method to detect PRGBS as the likelihood of this screen being more routinely performed increases due to the global rise in PRGBS identification.

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