National Meticillin-Resistant *Staphylococcus aureus*

Reference Laboratory

Annual Report, 2010

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Summary

The National MRSA Reference Laboratory (NMRSARL) supports efforts to prevent and control MRSA in Ireland by providing expertise to laboratories in the correct identification of *Staphylococcus aureus* isolates, by tracking circulating strains as part of infection control, by detecting the emergence of new mechanisms of resistance to antibiotics, by screening for the presence of novel virulence factors or toxins, and by participation in research and development initiatives at home and abroad.

During 2010, there were 280 cases of bloodstream infection due to MRSA, compared with 325 and 407 cases in 2009 and 2008, respectively. By providing timely and complete surveillance data, by supporting microbiology laboratories throughout the country and through its educational role in optimising the detection, identification and control of MRSA, the work of the NMRSARL has contributed to a decline in the proportion of *S. aureus* bloodstream infection due to MRSA. The proportion of *S. aureus* isolates recovered from blood cultures in Irish hospitals that participated in the European Antimicrobial Resistance Surveillance Network (EARS-Net) exhibiting meticillin resistance has decreased during the past two years from 42% in 2006 to 24.4% in 2010\(^1\). One particular strain type, AR06, predominates among blood-stream isolates accounting for 81.2% of isolates (227/280) investigated\(^2\).

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\(^2\)This report.

*, The spelling ‘meticillin’ is used in place of ‘methicillin’ in accordance with International Pharmacopoeia guidelines.
Reassuringly in 2010, there were no reports of vancomycin-resistant MRSA as vancomycin remains the drug of choice for the treatment of many MRSA infections. PVL toxin, which often indicates the presence of community-acquired MRSA, was detected in 19% of isolates investigated. The NMRSARL also assisted other laboratories on 15 occasions regarding laboratory aspects of MRSA and participated in the investigation of 34 outbreaks.

The NMRSARL expanded its service to include the investigation of meticillin-susceptible S. aureus (MSSA) isolates in outbreak situations and continued its national and international collaborations including in the areas of optimising the characterisation or typing of different strains of MRSA.

Despite restrictions on staff and funding, NMRSARL intends to continue to meet the needs of its users in the future and also enhance the safety of patient care by on-going analysis of strains of MRSA. In addition, NMRSARL aims to enhance services by further investigation of infection caused by MSSA strains as MSSA can cause the same serious illness as those caused by MRSA.

Dr. Brian O’Connell, Gráinne Brennan, Prof. Hilary Humphreys,
Director Chief Medical Scientist Honorary Consultant

*, The spelling ‘meticillin’ is used in place of ‘methicillin’ in accordance with International Pharmacopoeia guidelines.
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Background

The National Meticillin-Resistant Staphylococcus aureus (MRSA) Reference Laboratory (NMRSARL) was officially opened on 23\textsuperscript{rd} January, 2002. The NMRSARL is located in St. James’s Hospital (SJH) and works closely with the SJH Department of Microbiology, Central Pathology Laboratory. In 2010, funding for NMRSARL came from the Department of Health and Children (DoHC) through the Health Service Executive to SJH. Local administration is through SJH.

NMRSARL currently offers the following services to microbiology laboratory medical and scientific staff in hospitals in Ireland:

- confirmation of \textit{S. aureus} identity and meticillin resistance
- epidemiological typing using
  - antibiogram-resistogram (AR) typing
  - biotyping
  - DNA macrorestriction analysis using pulsed field gel electrophoresis (PFGE)
- investigation of glycopeptide resistance
- detection of virulence factors, at present, restricted to the Panton-Valentine leukocidin (PVL), a virulence factor associated with CA-MRSA causing skin and soft tissue infection and investigated in NMRSARL for research purposes only
- advice on treatment of patients with MRSA through its medical director
- advice on infection prevention and control through the infection control team of SJH
- advice on laboratory aspects of MRSA through the scientific staff of NMRSARL.

During 2010 in addition to the routine services in NMRSARL, staff validated the use of PFGE for the investigation of meticillin susceptible \textit{S. aureus} (MSSA) in outbreak situations and now provides this service to its users.

Current polymerase chain reaction (PCR) assays include detection of \textit{meca} (which encodes meticillin resistance) and \textit{pvl} (encoding PVL).

Part of the NMRSARL’s current work is the routine monitoring of MRSA strains prevalent in Ireland. This is done by AR typing, biotyping and PFGE typing of blood culture MRSA isolates from Irish hospitals that participate in the European Antimicrobial Resistance Surveillance Network (EARS-Net, previously EARSS).

In addition, NMRSARL is involved in research, education and training.
Achievements in 2010
NMRSARL Laboratory Work

European Antimicrobial Resistance Surveillance Network (EARS-Net)

Forty-two laboratories throughout Ireland submitted 280 blood-stream MRSA isolates to the NMRSARL during 2010 for monitoring of epidemiological types and resistance to clinically significant antimicrobials. This laboratory work is performed for the EARS-Net project, a European initiative that, in Ireland, is operated through the Health Protection Surveillance Centre (HPSC).

All MRSA isolates are frozen on receipt in the NMRSARL for storage and any further work that may be required. Minimum inhibitory concentration (MIC) determinations of oxacillin and screening isolates for reduced susceptibility to vancomycin with the E-test™ macro-method are performed. In addition, the NMRSARL provides HPSC with data on rates of resistance to other clinically useful antibiotics. Additional data generated from these isolates include agar screening for glycopeptide resistance and teicoplanin E-test™ macro-method determinations.

Fusidic Acid Resistance

Monitoring antimicrobial resistance in MRSA isolates investigated under the EARS-Net has shown that resistance to fusidic acid increased from <10% between 1999 and 2001 to 27% in 2006 and to 40% (112/280) in 2010.

This is a worrying development as fusidic acid remains a clinically useful antimicrobial for difficult to treat infections.

Mupirocin Resistance

Between January 2006 and December 2007, the proportion of MRSA isolates exhibiting high-level mupirocin resistance (MpR) increased to 2.9% compared with 1.4% between January 1999 and December 2006. During the later period of 2008 55% of MpR isolates (16/29) were an unfamiliar AR pattern which included resistance to the aminoglycosides gentamicin, kanamycin and tobramycin but with PFG-01 patterns which are associated with the AR06 AR type while 14% (4/29) were AR-PFG 06-011. In 2010, high-level mupirocin resistance was detected in 2.8% (8/280) of MRSA isolates from blood; 25% of these MpR isolates (2/8) were the ‘old’ AR-PFG types 13-00 or 14-00 (or variants of these patterns) while 75% were the ‘unfamiliar’ AR pattern with aminoglycoside resistance but with PFG-01 patterns associated with the AR06 AR type.

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This work suggests that mupirocin resistance may be becoming established in the most common strain that is circulating in Irish hospitals. If this is the case, then it will undoubtedly have a major impact on the success of decolonisation, a major strategy in preventing spread, as this is the agent of choice to eradicate nasal colonisation with MRSA. The NMRSARL alerted microbiologists and infection control teams across the country of this potential development and guidance on use of mupirocin.

**Glycopeptide Resistance**

The glycopeptides vancomycin and teicoplanin remain the mainstay for the treatment of serious infection caused by MRSA. There has been increasing concern about the development of resistance to these agents over recent years and laboratory detection of resistance is complex. NMRSARL uses a vancomycin agar screen and an experimental teicoplanin agar screen for preliminary investigation of reduced susceptibility to glycopeptide. NMRSARL also tests all EARS-Net MRSA isolates by a commercial MIC system (E-test) for both vancomycin and teicoplanin resistance using a macro-method which screens for hetero-glycopeptide resistant *S. aureus* (hGISA). Isolates yielding positive results in the E-test macro-method are confirmed as hGISA by population analysis profile-area under the curve (PAP-AUC) ratio determination. During 2010 no isolates exhibited reduced susceptibility to glycopeptides.

**Linezolid, Quinupristin/dalfopristin, Daptomycin and Tigecycline Susceptibility Testing**

Monitoring of these newer agents for treatment of MRSA infection is important, as resistance detection is rare and difficult with not all laboratories having the capability to perform appropriate tests. All EARS-Net MRSA isolates are tested against linezolid and in 2010, all were susceptible. During 2010, 100 MRSA isolates (from the EARS-Net study) were tested for susceptibility to quinupristin/dalfopristin, daptomycin and tigecycline by E-test MIC determination. In addition, these isolates were tested against quinupristin/dalfopristin and tigecycline by disk diffusion. All isolates were susceptible to quinupristin/dalfopristin, daptomycin and tigecycline with MICs ≤1mg/L.

**Molecular Epidemiological Typing**

**Pulsed Field Gel Electrophoresis (PFGE)**

During 2010, all EARS-Net MRSA isolates were typed by PFGE. As reported previously, in the NMRSARL, PFGE patterns are assigned a five-digit PFGE type (PFT) number which are grouped into apparently-related groups of PFTs according to the criteria of Tenover *et al.*

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3. Clinical and Laboratory Standards Institute. CLSI Documents M7-A8; M2-A10; M100-S19; 2009.
AR typing and PFGE typing results are combined to yield AR-PFG types. The continuing predominance of AR-PFG 06-01 isolates (which are susceptible to most antimicrobials on the AR typing panel) is resulting in AR typing becoming less useful and PFGE typing being required more extensively. PFGE patterns of AR-PFG 06-01 isolates have tended to be relatively homogeneous. When Irish laboratories first participated in EARS-Net in 1999, just seven PFGE patterns were detected among the AR-PFG 06-01 isolates investigated. However with the development of the PFGE method used the number of patterns recognised has significantly increased to over 180 PFTs. Since 2001 two patterns (PFTs 01018 and 01039) have predominated. This trend continued in 2010, with 44.5% (99/280) of isolates exhibiting one of three closely-related patterns, PFT 01018, 01039 and 01042, which occurred at frequencies of 20%, 15% and 9.6%, respectively).

spa Typing
As previously mentioned AR typing and PFGE are becoming less useful in the typing of MRSA isolates in Ireland\(^2\). spa typing recognises mutations or repeat insertion / deletion events that can cause changes in the polymorphic X region of the staphylococcal protein A gene (spa) gene. It has been shown that there is good concordance between spa typing and PFGE. In addition to routine methods NMRSARL has also used spa typing to type a selection of MRSA and MSSA isolates. The availability of MLST data associated with spa types on an online database allows the inference of MLST on isolates spa typed in NMRSARL. Currently NMRSARL investigates all PVL positive isolates along with isolates exhibiting new or unfamiliar AR patterns or pulsed field types. In using spa typing NMRSARL is able to compare Irish isolates with isolates from all other countries.

In 2010 42 PVL-positive isolates and 51 other S. aureus isolates were spa typed. Among PVL-positive isolates (Figure 1 Panel A), the predominant spa type was t657 (associated with ST772) detected among 20% of the isolates. Other spa types occurring in more than one isolate were t008 (ST8), t012 (ST30), t019 (ST30), t044 (ST80), t852 (ST22) and t3800 (ST30). Nine spa types occurred in only one isolate. Among the PVL negative isolates investigated there was only one isolate found for 22 spa types (Figure 1 Panel B). MLST data associated with spa types for which there was more than one isolate include ST5 ST8, ST22, ST30, ST88 and ST1430.

\(^2\) This report.
Figure 1: *spa* types exhibited by PVL-positive (Panel A) and PVL-negative (Panel B) isolates. *spa* types shown to the side are those exhibited by only one isolate.

MLST and SCCmec Typing

In 2002, the International Union of Microbiological Societies Subcommittee on Typing of Staphylococci agreed that a nomenclature based on MLST and SCCmec typing should be used for international comparison of MRSA strains. In previous reports, we showed that the majority of MRSA isolates recovered in Irish hospitals between 1971 and 2003 belonged to one of seven internationally spread MRSA clones (ST239, ST247, ST250, ST5, ST22, ST36 and ST8) and had SCCmec types I, Ia, II, III or IV but ST8 isolates showed an unexpected degree of diversity within the SCCmec element. The correlation between AR-PFG, MLST and SCCmec types is shown in Figure 3. Isolates with AR-PFG 06-01 exhibited MLST and SCCmec type ST22-MRSA-IV similar to UK EMRSA-15 while those with AR-PFG 07-02 were ST36-MRSA-II similar to UK EMRSA-16.

Monitoring the Epidemic Strains Prevalent in the Irish MRSA Population

In addition to data generated to support the EARS-Net programme in Ireland, NMRSARL investigates MRSA to monitor the epidemiological types of MRSA that are circulating in Irish hospitals. Most of the data presented in this report refers to blood-stream isolates that is important but not representative of the true burden of MRSA in Ireland. Isolates are typed by AR typing, biotyping and PFGE typing. Quarterly reports are issued to each participant laboratory detailing the overall AR type distribution among EARS-Net MRSA bloodstream isolates. NMRSARL also provides separate reports for each individual laboratory of that laboratory’s AR typing results. These results provide essential background information on the MRSA population in each hospital against which potential outbreaks can be assessed.

Since 2004, the NMRSARL has combined AR typing results with PFGE typing results to generate AR-PFG types\(^1\). Figure 3 shows how the AR-PFG type distribution in participating hospitals has changed since 1999 and also includes data on multilocus sequence typing (MLST) and staphylococcal cassette chromosome (SCC) \textit{mec} typing extrapolated from a study completed in 2004\(^2\). The prevalence of one AR-PFG type 06-01 increased from 22% in 1999 to 81.2% in 2010. Unlike other strains of MRSA, AR-PFG 06-01 isolates are not multi-antibiotic-resistant and, as this strain becomes increasingly predominant, AR typing and PFGE typing are becoming less helpful. Other typing methods are required to distinguish among AR-PFG 06-01 isolates.

**Figure 3.** Epidemiological type distribution among MRSA isolates in Irish hospitals that participate in the European Antimicrobial Resistance Surveillance Network (1999 to 2010). AR-PFG, antibiogram-resistogram type and pulsed field gel electrophoresis group; ST-SCC\textit{mec}, MLST sequence type and SCC\textit{mec} type (SCC\textit{mec} II variant, variants of SCC\textit{mec} type II).

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Detection of Virulence Factors
Panton-Valentine Leukocidin (PVL) Carriage in MRSA in Ireland
In a study investigating PVL in MRSA in Ireland, carriage of PVL was shown to be unreliable as a sole marker for community-acquired MRSA (CA-MRSA). Molecular characterization of PVL-positive MRSA isolates confirmed that all isolates carried SCCmec type IV but six genotypes (ST30, ST8, ST22, ST80, ST5 and ST154) were represented among these isolates. Epidemiological data showed that 36% of patients in Ireland from whom PVL-positive isolates were recovered were of non-Irish ethnic origin\(^1\). In 2010, 221 isolates were tested for carriage of PVL. These comprised 25 EARS-Net MRSA isolates (one was positive), 61 MSSA and 135 MRSA isolates. Eight percent of MSSA (5/61) and 26.7% of MRSA (36/135) were PVL-positive.

MRSA in Veterinary Medical Practice in Ireland
In 2010, NMRSARL continued its collaborative work with veterinary colleagues\(^2\).

Requests for Support and Assistance from Diagnostic Laboratories
In NMRSARL, all requests are considered incidents. Incidents may be requests for information, requests to investigate isolates from potential outbreaks or requests to investigate some other problem with the isolate(s). During 2010, the routine epidemiological typing service was limited to institutions experiencing acute problems and AR typing and biotyping only were routinely offered. Recognition of the problems created by the increasing prevalence of AR06 isolates resulted in increasing numbers of isolates requiring PFGE typing. All isolates were screened for glycopeptide resistance by agar screening.

In 2010, the NMRSARL processed:
- 280 isolates (from 272 patients) submitted under the EARS-Net project
- 15 requests for laboratory information regarding MRSA
- 200 requests for isolate investigation (on 416 isolates which included 34 outbreak investigation requests).

Workload figures for referral isolates (both bloodstream under the EARS-Net project and other isolates) are detailed in the table below. Overall, the number of incidents is increasing although overall numbers of blood-stream isolates have decreased.

Numbers of incidents, putative outbreaks and isolates investigated during 2009

<table>
<thead>
<tr>
<th>Year</th>
<th>Incidents</th>
<th>Isolates</th>
<th>Outbreaks</th>
<th>pvl</th>
<th>meCA</th>
<th>spa</th>
<th>PFGE</th>
<th>EARS-Net MRSA</th>
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<td>34</td>
<td>221</td>
<td>49</td>
<td>81</td>
<td>529*</td>
<td>280</td>
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</table>

*, all EARS-Net MRSA isolates (n = 280 in 2010) were investigated by PFGE and 35 were tested for pvl. pvl, Panton-Valentine leukocidin toxin gene; meCA, gene encoding meticillin resistance; spa, staphylococcal protein A gene typing; PFGE, pulsed field gel electrophoresis.

Quality Management System

The NMRSARL is fully accredited under Clinical Pathology Accreditation (CPA) standards incorporating ISO 15189. As part of the quality management system staff completed a number of internal and external audits and had a surveillance visit from CPA assessors. A number of minor non-conformities arising from these audits were corrected.

Although there is no appropriate external quality assurance scheme, which covers the work of the NMRSARL, staff in the laboratory have been working with Staphylococcal Reference Laboratories in Scotland, Copenhagen and London to develop an equivalent system. As part of an external quality assurance (EQA) exercise in 2010 NMRSARL submitted results of susceptibility testing, AR typing, PFGE and spa typing. In addition to this collaborators based in the Microbiology Unit of the Dublin Dental School provided results for SCCmeC typing and MLST.

Quality Objectives

As part of the Quality Management System the management of the NMRSARL set objectives for 2010 that involved all staff and were reviewed throughout the year. Along with quality issues these objectives cover areas relating to Staff Training and Education, Health and Safety, Waste Management and Service Development.
The NMRSARL recognises the increased demand from our users for the investigation of MSSA isolates both for the detection of *pvl* and for epidemiological typing. With this in mind NMRSARL aims to increase the availability of these services to our users in the future. As part of our service development during 2010 staff validated PFGE for use with MSSA isolates for outbreak investigation. An increase in *spa* typing of MSSA isolates has also led to a greater understanding of the strains circulating in Ireland.

**Resources**

**Staff**
In April 2010 Dr. Angela Rossney, Chief Medical Scientist retired from her post and the position remained vacant for the remainder of the year. The NMRSARL would like to acknowledge the huge contribution Dr. Rossney made to its establishment and subsequent success of the laboratory. Other staff employed at the NMRSARL included one staff grade medical scientist (Ms. Emma Gibbons), one molecular microbiologist (Dr. Pamela Morgan) and one medical laboratory aide (Mr. Paul Grier). A permanent position for a clerical officer had been sanctioned by DoHC but remained vacant. During 2010, a medical scientist (Ms. Gráinne Brennan) was seconded to the NMRSARL from the diagnostic Microbiology Department in SJH on a temporary basis and was appointed in an acting senior medical scientist capacity. The role of Director was discharged in an honorary capacity by Dr. Brian O’Connell, Consultant Microbiologist, SJH. In 2010, Professor Hilary Humphreys from the Royal College of Surgeons in Ireland and Beaumont Hospital was approached and agreed to take on the role of Honorary Consultant to provide an external perspective to the activities and services provided by the NMRSARL.

**Premises and Equipment**
NMRSARL consists of three main laboratory areas, a Phenotyping Laboratory, a Genotyping Laboratory and a PCR Laboratory. The provision of a suitable computer system is a major requirement, both for monitoring isolates received and for detailed analytical work. The Central Pathology Laboratory in SJH has been involved in procuring a new computer system for a number of years and as part of this procurement, the special requirements of NMRSARL have been noted. However, all systems investigated to date would require extensive modification to accommodate NMRSARL’s needs.

**Finance**
Funding of €300k was allocated for the year 2010. NMRSARL stayed within budget for the year.

**Committees**
NMRSARL staff members sit on the Irish EARS-Net Steering Committee and on the Royal College of Physicians of Ireland’s Policy Group on HealthCare-Associated Infection.
Education
NMRSARL plays a prominent role in education of laboratory staff, doctors and nurses throughout Ireland and is achieved by regular feedback, presentations and reports. In particular, NMRSARL staff gave lectures to microbiology students in the Moyne Institute, TCD and also made oral presentations at SJH Grand Rounds on ‘MRSA in the Surgical Patient’. Scientific staff demonstrated techniques used in NMRSARL to staff from other hospital laboratories, research facilities and transition year students.

NMRSARL staff completed internal training courses in areas such as fire safety, chemical safety, computer skills, the quality management system and journal clubs organized within the microbiology department of St. James’s Hospital. Staff also attended national and international conferences such as Biomedica, Dublin and the International Symposium on Staphylococci and Staphylococcal Infections, Bath.

Research
On-going research projects within NMRSARL include:

1) The monitoring of reduced susceptibility to glycopeptide using a new teicoplanin agar screen method, to investigate the role of teicoplanin population analysis profiling confirmation of reduced susceptibility to glycopeptide¹.

2) The monitoring of gentamicin-resistant MSSA (GentR MSSA) isolates recovered in SJH. A PVL-positive subset of these isolates with spa type t005 (associated with ST22) has been recognized in a number of European countries including Ireland

3) The development of a triplex real-time PCR assay for the detection of nuc, meca and pvl in MRSA and MSSA isolates

4) Replacing current North American Clinical and Laboratory Standards Institute (CLSI) susceptibility techniques in NMRSARL with European Committee on Antimicrobial Susceptibility Testing (EUCAST) techniques and determining the implication this change will have on AR patterns.

- The work described below has been undertaken in collaboration with Professor David Coleman and his team at the Dublin Dental School, TCD:

1) On-going investigation of remnants of SCCmec found in GentR MSSA²

2) Following a study investigating genotypes of the predominant strains of MRSA in Ireland, research is now focusing on MLST and SCCmec elements in infrequently-occurring, ‘sporadic’ and/or unusual MRSA isolates

3) Investigation of MpR isolates exhibiting AR type AR06 and the ‘unfamiliar’ AR pattern with aminoglycoside resistance but with PFG 01 patterns associated with the AR06 AR type
4) The identification of a new SCCmec element in MRSA isolates from Irish hospitals\(^1\)
5) The role of a S. aureus DNA array to further characterize predominant strains of MRSA in Ireland.

- Since 2005, the NMRSARL has been collaborating with Professors Hilary Humphreys (RCSI/Beaumont) and David Coleman (TCD) in an MRSA Translation Research Project funded by the Health Research Board. In on-going collaborative work with Professor Richard Goering, Creighton University, Nebraska, USA, the value of dru typing for distinguishing among AR-PFG 06-01 MRSA isolates recovered in Beaumont Hospital during this project has been undertaken\(^2,3\). Following an evaluation by NMRSARL of a rapid commercial real-time PCR technique (the Xpert MRSA assay, Cepheid) for detection of MRSA in screening specimens, the impact of the use of this system in clinical practice to reduce time from patient admission to isolation was assessed in Beaumont Hospital\(^4\).

**International Collaboration**

NMRSARL is a member of the Harmony Group (an international group set up to standardize (‘harmonise’) PFGE methods). Between June 2005 and May 2007, NMRSARL participated in a UK / Irish study of MRSA bloodstream infection in paediatric patients\(^5\). The NMRSARL participated in the EARS-Net spa typing project and the results of the work were published in January 2010\(^6\). Collaborative work with Professor Richard Goering, Creighton University, Nebraska, USA proved fruitful in providing a potentially discriminatory method for distinguishing among AR-PFG 06-01 MRSA. Discussions with staff from the Scottish, Danish and UK Reference Laboratories to organize an external quality assessment (EQA) programme between the four centers continue. To date there have been three distributions since September 2007.

**Collaboration with Other Irish Reference Laboratories**

NMRSARL maintains contact with colleagues in other Irish Reference Laboratories such as the Meningococcal Reference Laboratory.

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\(^2\) Rossney AS, Goering RV. 17th ECCMID, Munich, Germany, 31 March–03 April 2007.
\(^3\) Shore AC, Kinnevey P, Rossney AS et al. 13th ISSSI; Cairns, Australia, 2008. 7–10\(^{\text{th}}\) September 2008.
\(^5\) Goodall CM, Johnson AP, Sharland M et al. *Archives of Diseases in Childhood* 2009 In press.
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Articles


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