by his hyperglycaemia. Impaired renal function reduces glucose loss via the kidney, further leading to elevation in blood glucose level.

As DKA and HHS can occur in both diagnosed and undiagnosed diabetic patients, it can also be complicated by hypertension and functional renal failure. Therefore, during the treatment of hyperglycaemia, underlying causes other than diabetes should be investigated.

When phaeochromocytoma is considered, appropriate approaches must be used for its diagnosis and localisation. Measurement of plasma metanephrine is the biochemical test of choice. A three- to four-fold elevation of plasma metanephrine is associated with nearly 100% specificity for the diagnosis of phaeochromocytoma in patients without renal failure.[9] When available, the measurement of plasma free metanephrine should be performed. In sporadic phaeochromocytoma, the test has a reported sensitivity of 99% and a specificity of 82%.[10]

Once phaeochromocytoma has been diagnosed, appropriate antihypertensive drugs are used to manage hypertension, control the associated cardiovascular symptoms and prepare the patient for surgery.

In conclusion, although uncommon, the diagnosis of phaeochromocytoma should be considered in patients with severe hypertension complicated by acute renal failure, acidosis and hyperglycaemia. □

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Bovine serum albumin reduces effects of endogenous inhibitors in transport media, facilitating real-time PCR detection of methicillin-resistant *Staphylococcus aureus* from screening swabs

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Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers by conventional culture techniques requires at least 48 hours to complete, is labour intensive and frequently is a significant drain on laboratory resources. In contrast, real-time polymerase chain reaction (RT-PCR) technology offers 'same day' accurate identification of MRSA, representing a significant improvement in turnaround times.[1–4] Crucial steps for laboratory molecular diagnosis of MRSA include adequate collection and transport of various screening specimens available (e.g., anterior nares, perineum, groin), rapid and efficient release of target DNA, and elimination of potential PCR inhibitors.[5]

The vast majority of screening specimens sent for routine analysis to UK laboratories are collected on cotton-tipped swabs which are placed subsequently in a non-nutritive medium (e.g., Amies) and transported to the laboratory. Such collection systems are simple, cost-effective and easy to use, and it would be advantageous to retain this specimen collection format combined with efficient DNA preparation for rapid RT-PCR testing. However, it is known that swabs containing calcium alginate[6,7] and those with aluminium shafts[7] are inhibitory to PCR. Additionally, agar that survives the DNA preparation stage is known to suppress PCR amplification.[8]

In contrast to commercially available column purification extraction techniques (which theoretically provide samples containing the least amount of PCR inhibitory substances), simple bacterial lysis has the ability to provide bacterial target DNA rapidly for analysis, is cost effective and has high-throughput potential, making it an ideal choice for a busy diagnostic laboratory.[4,9]

During development of an in-house real-time SYBR Green 'dual locus' PCR assay targeting *nuc* and *mecA* genes, it was observed that after lysis of specimens a component of Amies transport medium was inhibiting PCR detection of MRSA, leading to false-negative results. An alternative approach to circumvent PCR inhibition is to enhance or facilitate PCR amplification.[10]

Reports indicate that addition of a suitable PCR facilitator such as non-acetylated bovine serum albumin (BSA) to RT-PCR mixes can relieve the effects of inhibition.[10–15] This study reports the use of non-acetylated BSA as a PCR facilitator, enabling the real-time detection of MRSA directly from clinical screening swabs.

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Swabs were prepared for PCR using a cell lysis method based on that described previously.[4] Briefly, a swab was broken off into a microcentrifuge tube containing 200 µL lysis buffer (10 mmol/L Tris-HCL [pH 8.0], 1mmol/L EDTA containing 1U/µL achromopeptidase [Sigma Chemical Co.]). Lysis solution containing swabs was vortex-mixed for 10 sec and incubated at 37°C for 20 min. The sample was then boiled for 5 min and then centrifuged at 10,000 x*g* for 1 min. The lysis solution was removed carefully from the tube using a 1000 µL pipette tip, and transferred into a fresh microcentrifuge tube and stored at –80°C until required. An extraction control consisting of an uninoculated swab was extracted with each batch of specimens.

Seventeen known MRSA culture-positive screening swabs (six perineum, seven nasal, four groin) and resultant positive screening plates were obtained from the routine microbiology laboratory. Swabs were processed as outlined. The screening plates were graded arbitrarily as follows: low (<50 MRSA colonies), medium (50–100 MRSA colonies) or high (>100 MRSA colonies) MRSA presence.

A 10-fold serial dilution of an MRSA clinical isolate was performed in sterile saline. Colony forming units (cfu)/mL where determined by the spread plate method[16] on Columbia blood agar (CBA) and carried out in triplicate for each dilution. The CBA plates were incubated at 37°C overnight and examined the next day and up to 48 h. A 20 µL sample of each dilution series was used to spike Amies transport swabs (Sterilin, UK) in duplicate by careful application to a cotton swab using a pipette tip. Swabs with 20 µL sterile saline added acted as negative controls.

All swabs were stored overnight at 4°C before processing. The next day, one swab from each dilution (growth control) was streaked on CBA to determine the presence of viable MRSA. The CBA plates were incubated at 37°C overnight and examined the next day. The remaining swabs were subjected to the lysis procedure outlined previously.

The RT-PCR method was accomplished using the ABI7000 sequence detection system (Applied Biosystems). Oligonucleotide primers for the *nuc* gene were designed in-house ([forward primer] Sau5A: 5′ CAAGTCTAAGTAGCTCAGCAAATG 3′ and [reverse primer] Sau5B: 5′ AC[C/A]GTATCACCATCAATCGC 3′ yielding a 188-bp fragment). Primers for the *mecA* gene were based on those described previously,[17] with some modification ([forward primer] Mec2A; 5′ CTAGGTGTTGGTGAAGATATACCA and [reverse primer] Mec2B: 5′ TGAGGTGCGTTAATATTGCCA yielding a 150-bp fragment).

All primers were synthesised by operon and resuspended in TE buffer (Promega) prior to use. Lysed specimen (2 µL) was added to 8 µL of appropriate real-time *mecA* and *nuc* PCR mastermixes. Positive controls were lysed MRSA DNA preparations stored at –80°C. A no-template control (NTC) consisting of 2-µL nuclease-free water (Promega) was included. All specimens and controls were tested in duplicate for each mastermix. The final 10-µL 1x reaction mix (with varying BSA concentrations) contained 1xPCR buffer (Promega), 0.2 mmol/L NTP (Promega), 3.5 mmol/L $MgCl^2$ (Promega), 0.2 µmol primers (Operon), 1x SYBR Green (Sigma), 500 nmol ROX (Invitrogen), 0.25 units *Thermus aquaticus* (*Taq*) polymerase (Promega), and 1, 2 or 4 µg BSA (Sigma).

Cycling parameters were as follows: 94°C for 3 min, followed by amplification for 40 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 30 sec, with fluorescence acquisition after each cycle followed by a standard melt programme.

Table 1. MRSA (cfu/20 µL) added to Amies transport swabs and subsequent culture positivity rates of inoculated swabs following overnight incubation at 4°C.

cfu/20 µL	Culture result
2.2×10^5	+
2.2×10^4	+
2.2×10^3	+
220	+
22	–
Negative control	–

The identity of the PCR product from each sample was confirmed by analysing melting temperature (T_m) and comparing it with the relevant positive control. If T_m was within ~1.0°C of the positive control for both *mecA* and *nuc* products then the sample was regarded as positive.

Table 1 shows the cfu/mL for 10-fold serial dilutions carried out for MRSA and subsequent culturability of 20-µL inoculated swabs for each dilution of MRSA retained at 4°C overnight. Endpoint detection of MRSA, as measured by the presence of >1 colony on an overnight CBA plate, was 220 cfu per spiked swab.

The effect of increasing BSA concentration in RT-PCR mastermixes compared to no BSA for the detection of spiked MRSA Amies transport swabs is shown in Table 2. The use of BSA at a final concentration ≥2 µg/µL PCR mastermix resulted in an improvement in the PCR amplification of *mecA* and *nuc* genes from MRSA-spiked Amies swabs, allowing detection of 2.2×10^4 cfu MRSA per swab (this represents a total of 220 cfu MRSA per 2 µL analysed in the final PCR reaction mix). The use of BSA concentrations <2 µg/µL RT-PCR mastermix had no effect on the PCR amplification of *mecA* and *nuc* genes from MRSA-spiked Amies swabs.

The use of MRSA RT-PCR mastermix containing a final concentration of 2 µg/µL BSA versus an RT-PCR mastermix

Table 2. Real-time MRSA PCR results for spiked Amies transport swabs: comparison of four mastermixes. Included are MRSA DNA positive control, negative (saline-spiked swab) control and no-template control (NTC).

Sample	cfu/20 µL (per swab)	No BSA	1 µg BSA /PCR	2 µg BSA /PCR	4 µg BSA /PCR
10^{-1}	2.2×10^5	–	–	+	+
10^{-2}	2.2×10^4	–	–	+	+
10^{-3}	2.2×10^3	–	–	–	–
10^{-4}	220	–	–	–	–
Negative control	–	–	–	–	–
MRSA-positive	–	+	+	+	+
No-template control	–	–	–	–	–

Table 3. Evaluation of real-time PCR mastermix containing a final concentration of 2 $\mu g/\mu L$ BSA versus real-time PCR mastermix containing no BSA for the detection of MRSA from 17 MRSA culture-positive clinical screening swabs. Level of MRSA on each screening plate is included.

Clinical swab sample	Level of MRSA on screening plate	Mastermix (no BSA)	Mastermix (+2 µg BSA)
#1	Medium	–	+
#2	Medium	–	+
#3	Low	–	–
#4	Medium	–	+
#5	Medium	–	+
#6	Medium	–	+
#7	Medium	–	+
#8	Low	–	–
#9	Medium	–	+
#10	Low	–	+
#11	Medium	–	+
#12	High	+	+
#13	Medium	+	+
#14	Low	–	+
#15	Medium	–	+
#16	Low	–	+
#17	Medium	–	+
MRSA-postive DNA preparation	–	+	+
Negative control	–	–	–
No-template control	–	–	–

without BSA was evaluated for 17 known MRSA culture-positive clinical screening swabs. The swabs had undergone bacterial lysis preparation as outlined above. The results (Table 3) also included relevant MRSA colony levels scored for each screening plate. The mastermix containing BSA enabled the detection of 15/17 swabs (88%) compared to 2/17 swabs (11.8%) detected using a mastermix without the addition of BSA.

As RT-PCR becomes accepted in the molecular diagnosis of MRSA, attention must be given to specimen collection systems that are compatible with available extraction and/or lysis protocols. Ultimately, the usefulness of RT-PCR will be determined by the ability to obtain sufficient template DNA for analysis, combined with a reduction in the presence of PCR inhibitors. Amies swabs represent the most widely used transport system for current screening of hospitalised patients and it would be advantageous to adapt any molecular methods to this existing scheme.

Various organic and non-organic products are known to interfere with PCR reactions on several levels, particularly with *Taq* polymerase, leading to attenuation or complete inhibition.[10] Swabs and transport media have been shown to contain components that inhibit or suppress PCR, including calcium alginate,[6,7] aluminium shafts[7] and agar.[8]

Previous reports that looked at the use of RT-PCR to detect MRSA from transport swabs have relied on a range of commercial DNA extraction kits,[2,3] which can prove expensive and may increase hands-on time, thus reducing their usefulness in the busy microbiology laboratory.[9] A rapid and inexpensive lysis system has been used successfully to obtain MRSA template DNA from nasal swab specimens for subsequent diagnosis by RT-PCR.[4] This rapid lysis system was chosen for use in the authors' laboratory in conjunction with an in-house MRSA RT-PCR SYBR Green assay targeting *mecA* and *nuc* genes.

Initial findings disagreed with those obtained by Paule *et al*.,[4] and seemed to confirm those of Gibb and Wong,[8] which indicated that agar from transport medium present in lysates was suppressing DNA amplification. It has been reported that the addition of suitable PCR-enhancing agents such as non-acetylated BSA in various concentrations represents a potential cost-effective method for overcoming PCR amplification inhibition.[10–15] Additionally, Paule *et al*.[4] employed an RT-PCR Light Cycler (Roche Applied Science) to detect MRSA from transport swab lysates. A common component of RT-PCR Light Cycler mastermixes is BSA, which is used to prevent adherence of target DNA to glass capillaries used in such systems. This could explain why these authors reported no PCR inhibition because BSA inadvertently minimised the effects of inhibitors. Human serum albumin is adept at binding to a wide variety of molecules,[18] and presumably BSA functions in much the same manner, binding to and neutralising PCR inhibitors.

The RT-PCR detection of MRSA from spiked screening swabs employing a series of mastermixes containing a final concentration of 1 µg, 2 µg and 4 µg non-acetylated BSA versus mastermix without BSA was investigated in this study. Results showed that addition of BSA at a final concentration of ≥2 µg/µL mastermix resulted in an end-point detection of 2.2 x 10^4 cfu MRSA per spiked swab compared to non-detection when using <2 µg BSA or no BSA. End-point detection based on the ability to culture spiked swabs stored at 4 °C overnight on CBA revealed a cut-off limit of 220 cfu MRSA, a difference of 2 logs compared to RT-PCR detection. However, when taking into consideration the fact that 2-µL swab extract was analysed per PCR reaction, the cut-off for RT-PCR equates to 220 cfu MRSA. The 10^{-5} dilution of MRSA-spiked swab was not detected by culture the next day. This swab was estimated to contain 22 cfu, which, given the small number of MRSA present and the potential for cells to be sloughed into the surrounding transport medium, is not surprising.

Given that a minimum concentration of 2 µg BSA/µL facilitated PCR on spiked swabs, the application of real-time mastermixes containing this concentration of BSA versus no BSA was then evaluated on 17 clinical screening swabs. The mastermix containing BSA resulted in RT-PCR detection of 15/17 swabs (88%) compared to 2/17 swabs (11.8%) when using a mastermix without BSA addition. Total time from swab preparation to final PCR result was approximately three hours (~30 min for lysis, ~2.5 h for PCR).

Two swabs were missed by RT-PCR. These swabs were 'secondhand' (i.e., they had been streaked on MRSA-selective media for clinical evaluation beforehand) and this could be a possible explanation for the negative RT-PCR results. Retrospective examination of corresponding screening plates for each swab revealed the presence of two identifiable MRSA colonies on plate #8 and six colonies on screening plate #3. This indicates an initial low level of MRSA cells on the screening swabs, which could have been

removed by prior streaking on selective plates, resulting in a sample containing little or no target DNA for molecular analysis.

In summary, the results of this study indicate that direct DNA release through bacterial lysis, combined with the addition of ≥2 μg BSA, represents an effective system for the RT-PCR detection of MRSA from screening swabs, and potentially could be applied to similar pathogen transport systems. In addition, the authors suggest that equipment used for specimen collection, transport and processing should be accessed and optimised for RT-PCR assays. □

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Prevalence of *Salmonella typhi* among food handlers from bukkas in Nigeria

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Salmonella enterica serovar *typhi* is the aetiological agent of typhoid fever and causes an estimated 16.6 million cases and 600,000 deaths worldwide each year. A syndrome similar to typhoid fever is caused by paratyphoid serotypes of *Salmonella* species.[1]

Salmonella typhi is transmitted through food or water contaminated with faeces from infected persons, persistent excretors or from chronic asymptomatic carriers who handle food. Humans are the only host for *S. typhi* and there are no known environmental reservoirs.[1,2] Typhoid fever remains a major public health problem in many developing countries.[2] It is a sporadic disease in developed countries, occurring mainly in travellers returning from overseas. It can also produce the occasional point-source epidemic.[3]

In endemic areas, identified risk factors for the disease include eating food prepared outside the home (e.g., ice cream, flavoured iced drinks) by street vendors,[4] drinking contaminated water,[5] close contact with an infected person,[6] poor housing with inadequate facilities for personal hygiene,[7] and the recent use of antimicrobial drugs.[4]

Typhoid fever is among the major widespread diseases affecting the population in Nigeria and has been rated eighth among these common infections.[8] Nigeria, like many other tropical and developing countries, has been described as an endemic zone for typhoid fever.[9,10]

In Nigeria, transmission of typhoid fever occurs all year round but rates are slightly higher in April and July, coinciding with the height of the hot, dry season and the onset of the rainy season, respectively.[11] The highest number of cases of typhoid fever are recorded during the rainy season in south-east Nigeria.[10] Typhoid fever has been reported in all age groups and classes in Nigeria.[12] Owing to the irregular nature of bacterial shedding, several samples

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