Tropical Biomedicine 34(3): 648–656 (2017)

Secular trends of *Streptococcus pyogenes* sepsis in Pakistan and analysis of clinical features in a hospitalized cohort

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Received 18 September 2016; received in revised form 22 May 2017; accepted 24 May 2017

Abstract. *Streptococcus pyogenes* or Group A *Streptococcus* (GAS) is a re-emerging pathogen of significant public health importance. We present trends in GAS blood cultures over a 10 year period in Pakistan and characteristics of hospitalized patients with GAS sepsis over three years at a tertiary care hospital in Karachi, Pakistan. Blood cultures positive for GAS from 2004 -2014 were recorded at the clinical microbiology laboratory of the Aga Khan University and studied for trends in positivity rates. Medical records of patients hospitalized at the Aga Khan University hospital from 2012-2014 were also examined for clinical features and outcomes. GAS trends show a steady rate of blood culture positivity over 11 years, with higher rates in those >50 years, and seasonality favouring winter months. Case fatality rate in the hospitalized cohort was 34.1% (n= 14; of 41 patients). Malignancy predominated as the underlying predisposing factor among the 15-49 age group. Presence of sepsis was an independent predictor of mortality in GAS bacteremia. Studies of GAS trends in developing regions are important to inform changing epidemiology. GAS septic shock continues to have high case fatality despite antibiotic treatment. Early recognition, aggressive, goal-directed therapy for sepsis and prevention are possible control measures to prevent high mortality.

INTRODUCTION

Streptococcus pyogenes or Group A Streptococcus (GAS) bacteremia and sepsis are associated with a high mortality. Recent increase in the burden of GAS sepsis has been observed in USA and Australia, while high rates continue to be reported from several resource-limited settings (Efstratiou & Lamagni, 2016). While outbreaks associated with virulent strains are responsible for the upsurge seen in the community (Athey *et al.*, 2016), the spectrum of disease has shifted among hospitalized patients such that higher rates are observed in immunosuppressed and debilitated patients.

Pakistan has a high prevalence of diseases predisposing to GAS sepsis such as diabetes and malignancies (Guariguata *et al.*, 2014; Bhurgri *et al.*, 2011). Furthermore, this region also has a high burden of rheumatic heart disease, a notable sequel of GAS infection (Seckeler & Hoke, 2011). Despite these indicators of a high burden of both invasive and non-invasive GAS infection, no data on GAS bacteremia is available from Pakistan, and GAS sepsis remains understudied and unpublished from this region. We present trends of GAS bacteremia over the last 11 years based on blood culture positivity rates at a major diagnostic laboratory in Karachi, Pakistan. We have further analysed 41 cases of GAS bacteremia admitted at a tertiary care centre in Karachi for clinical features and outcome with a view to inform the recent epidemiology of GAS sepsis in southern Pakistan.

METHODS

Setting

The study was carried out at the Aga Khan University (AKU), Karachi, Pakistan. The university has a medical college with an affiliated 560-bedded tertiary care teaching hospital. The clinical laboratory has a network of over 200 collection points all over Pakistan. While the laboratory information system archives data on patient age and results for 10 years, no clinical data is recorded in the laboratory database, therefore examination of clinical features in all patients was not possible. The hospital records are archived online for 6 years, however, information was retrievable for 3 years and therefore clinical features among GAS sepsis patients were studied for a 3 year period.

Microbiological methods

The clinical laboratory uses BACTEC 9240 system for automated blood culture and this was used throughout the study years. Positive cultures were processed as per recommendations by the American Society for Microbiology (Garcia & Isenberg 2007), and susceptibility testing was performed according to the Clinical Laboratory Standards Institute guidelines (Clinical Laboratory Standards Institute 2003).

GAS bacteremia trends 2004-2014

Data was retrieved from the existing laboratory data sources. The AKU Information and Laboratory Management System (ILMS) software records and saves blood culture volumes and results of all samples processed at the AKU clinical microbiology laboratory. Laboratory data of blood cultures positive for *S.pyogenes* from individuals of all ages was retrieved from ILMS from 2004 to 2014 and entered into MS Excel. Frequencies were calculated and trends determined in Microsoft Excel.

GAS sepsis clinical features

Medical records of patients admitted to the Aga Khan University Hospital from 2012 to 2014 with blood cultures positive for GAS were reviewed for information on presenting illness (focus of infection and presence or absence of shock), comorbids, antibiotics administered, duration of hospital stay, and outcome of illness upon discharge.

Definitions: Bacteremia without a focus of infection was classified as 'occult'. Sepsis in children 0-14 years of age was defined as any two of: tachycardia, tachypnea or temperature instability accompanied by systolic blood pressure (SBP) less than the American Heart Association (AHA)'s fifth percentile SBP for age (i.e. newborn 60 mmHg, infant 70 mmHg, 1-10 years 70 (+ twice the age in years) mmHg, and in those 10 years or more 90 mmHg) (Goldstein *et al.*, 2005). Sepsis in those older than 14 years was defined according to the 2015 Sepsis 3.0 definitions, i.e. at a minimum a Sequential Organ Failure Assessment (SOFA) score of 2 or more, or presence of quick SOFA (qSOFA) signs (SBP <100 mmHg, respiratory rate of 22/min, altered mental status) (Singer *et al.*, 2016). Antibiotic treatment received at presentation was defined as "appropriate" (Harbarth *et al.*, 2007) if it included a beta lactam agent with known GAS activity (imipenem or meropenem, piperacillin, ampicillin, amoxicillin with or without a beta lactamase inhibitor, ceftriaxone, cefotaxime) (Areas *et al.*, 2014) with or without vancomycin, or clindamycin. "Time-to-antibiotic" was defined as the interval between the patient's arrival to the emergency department (ED) and the administration of the first dose of antibiotic as reflected by the time entered in the online pharmacy system.

Frequencies and proportions were calculated in MS Excel. For comparison of proportions or median times, when appropriate, Students t test or the Mann-Whitney-U test were used, and p values of <0.05 were considered significant. Malignancy, clindamycin adjunctive therapy, sepsis, door-to-antibiotic time and duration of hospital stay as predictors of mortality on hospital discharge were analysed with a binary logistic regression model with outcome (death) as a dependent variable. Statistical analyses including logistic regression analysis were carried out in SPSSv 19.0 (Armonk, NY: IBM Corp).

RESULTS

GAS bacteremia trends

Over 11 years, blood culture GAS positivity rates remained stable (Figure 1). Of 416451 blood cultures processed from 2004 to 2014, 200 (0.05%) blood cultures were positive for GAS. Mean age of patients in this series was 45.1 ± 25.7 years. Episodes of GAS bacteremia were observed more frequently in winter months where cases steadily increased in the October-March period and declined in the April-September period (Figure 2a). When disaggregated by age, highest numbers of cases were observed in those older than 50 years (Figure 2b). Number of cases in those older than 50 years was seen rising with a periodicity, with an associated decrease in the number of cases in the paediatric age group.

Resistance rates for chloramphenicol, ofloxacin, erythromycin and clindamycin varied over 11 years, with no established trends. Overall proportion of chloramphenicol, ofloxacin, erythromycin and clindamycin resistant strains was 18.3%, 5.5%, 29.5%, and 25.8%, respectively.

Clinical features in a 3 year hospital cohort

Over 3 years, 41 cases of GAS bacteremia were treated at the Aga Khan University Hospital in Karachi. Male to female ratio was 3:4. Median age of patients was 61 years [IQR 70-35], with 6 patients in the paediatric age group (0-14 years), 9 patients in the 15-49 age group and 26 patients over 50 years of age. Clinical features are described according to age groups and outcome of bacteremia (recovery vs death) in Table 1.

Site of infection: Cellulitis of the limbs was the most common focal site of infection. The incidence of occult bacteremia was 29.3% (n=12), and malignancy was a common underlying condition in the 15-49 years age group (66.7%, n=6). Upon presentation to the ED, 41.5% (n=17) patients had sepsis, and 8 of these had septic shock. Toxic Shock Syndrome (TSS) was present in one child with erysipelas.

Mortality and risk factors: Of 41 patients, 14 (34.1%) patients with GAS bacteremia died. Among patients with sepsis at presentation (n=18, 43.9%), most did not survive (n=12, 66.7%). The overall duration of hospital stay was significantly shorter

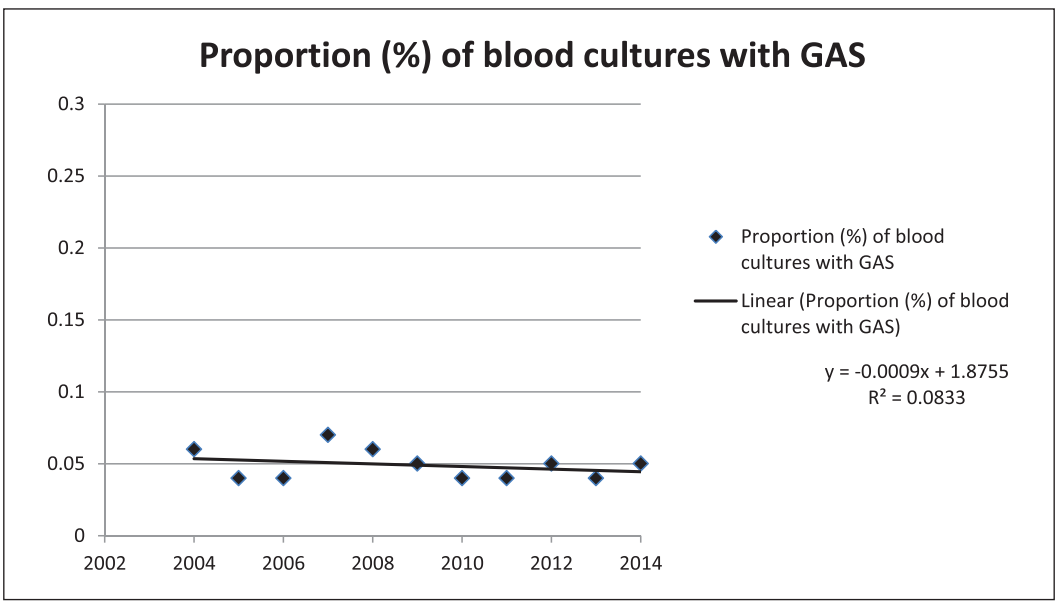

Figure 1. GAS blood culture positivity rates at the Aga Khan University Clinical Microbiology laboratory from 2004-2014.

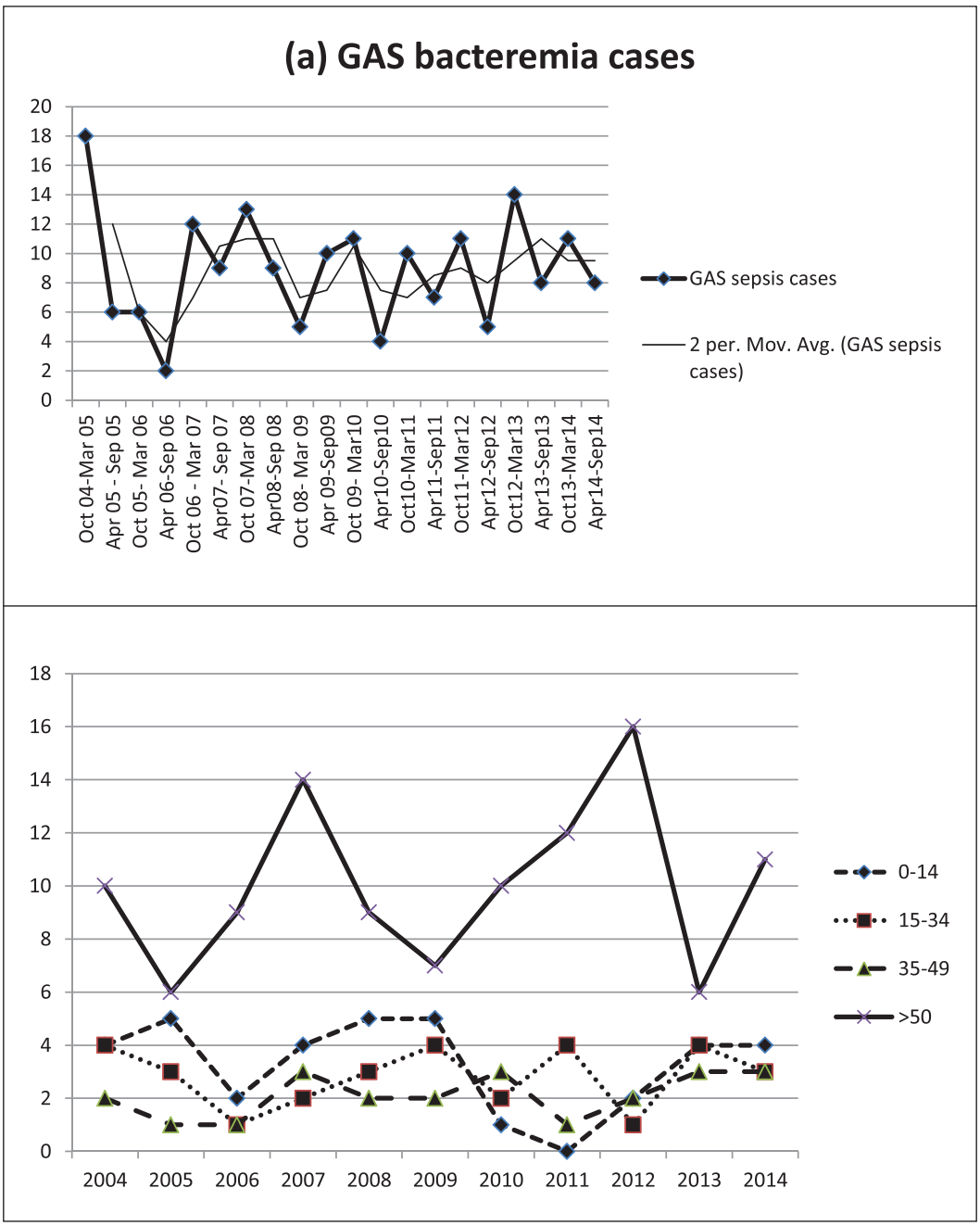

Figure 2. Seasonality of GAS bacteremia. A rise in winter months is observed (a); Trends in age groups show that those >50 years are the largest group affected (b).

among those who died (median [IQR] of 1.5 [3-1] days) than among survivors (median [IQR] of 4 [7-2] days); with p=0.04 (Mann-Whitney U test) as shown in Table 2. Lactate levels were performed in 12 patients. Patients with levels of 2 mmol/L or more within the first 24 hours of hospital admission did not survive (Table 2). Other risk factors for mortality are also shown in Table 2.

Empiric antibiotics and de-escalation: All patients received an appropriate beta lactam as part of their antibiotic regimen.

Table 1. Baseline characteristics by age groups of 41 patients with GAS bacteremia at the Aga Khan University Hospital from 2012 to 2014

	AGE GROUPS						TOTAL
	0–14		15–49		≥50		
	Recovered	Died	Recovered	Died	Recovered	Died	
Outcome	6	0	6	3	15	11	41
GAS source (% community acquired)	6 (100%)		3 (50%)	1 (33.3%)	13 (100%)	6 (60%)	28/41
Focus of infection	2 occult, 2 Fournier's, 1 septic arthritis, 1 erysipelas (trunk)		2 LL cellulitis, 1 UL cellulitis, 2 occult, 1 septic abortion	2 pneumonia, 1 UL cellulitis	5 occult, 5 LL cellulitis, 4 UL cellulitis, 1 diabetic foot infection	3 LL cellulitis, 1 UL cellulitis, 3 occult, 1 Fournier's, 1 septic arthritis, 1 orbital cellulitis, 1 SBP	
Sepsis at presentation	1		2	2	3	10	18
Shock at presentation	0		1	1	1	7	10
Underlying illness							
Malignancy			4	2	1	5	12
Chronic disease					DM = 9* Chronic kidney disease=1	2 (IHD=1; HCV=1)	12
Other	Diabetes insipidus (n=1; 16.7%)		1 (Sickle cell anemia)	1 (rheumatic valvulitis)	Hypothyroidism 1		4
None	5		1		3	4	13
TOTAL							**41**

Table 2. Risk factors for GAS bacteremia and effect on mortality in 41 patients 2012-2015 at the Aga Khan University, Karachi

	Total n (%)	Survivals n (%)	Mortalities n (%)	P value
Total number	41 (100)	27 (65.9)	14 (34.1)	–
Age, Median [IQR]	61 [70-35]	51 [69-30]	66.5 [71-55]	NS
Male	18 (43.9)	10 (37)	8 (57)	NS
Diabetes	10 (24.4)	10 (37.4)	0	0.0088
Malignancy	12 (29.3)	5 (18.5)	7 (50)	0.03
No comorbidities	13 (31.7)	9 (33.3)	4 (28.6)	NS
Healthcare-associated	13 (31.7)	6 (22.2)	7 (50)	NS
Community-acquired	28 (68.3)	22 (81.5)	6 (42.9)	0.01
Occult	12 (29.3)	9	3	NS
Limb cellulitis	17	12	5	NS
Sepsis at presentation	17	5	12	0.000
Shock at presentation	10	2	8	0.00044
Duration of hospital stay (days), Median [IQR]	3 [7-1]	4 [7-2]	1.5 [3-1]	0.04
Time to antibiotics (minutes), Median [IQR]	60 [148.5-21]	60 [165-19]	52 [114-24]	NS
Lactate >2mmol/L in 24 hours	7/13	0	7 (53.8)	0.00054
Clindamycin as adjunctive therapy	8 (19.5)	8/27 (29.6)	0	0.02

More than half (68.3%; n=28) of patients received meropenem or piperacillin/tazobactam with or without vancomycin in the emergency room as broad spectrum therapy in sepsis. In 2 patients, vancomycin alone was used as primary therapy owing to penicillin allergy, both of whom improved and were discharged. Ceftriaxone was used in 10 patients while amoxicillin with clavulanate was used in one patient with cellulitis. Once culture results became available, antibiotic therapy was deescalated to intravenous benzylpenicillin in only 4 patients (2 patients improved and were discharged, one patient was transferred out, and one patient expired). None of the 41 patients received immunoglobulin. Clindamycin was added to primary beta lactam or vancomycin treatment in 19.5% (n=8) patients (all survived), and its use was significantly associated with survival (Table 2). Time to administration of antibiotics was observed to be more than the recommended 1 hour for sepsis except for the pediatric age group.

Predictors of mortality: The presence of sepsis with GAS bacteremia increased the likelihood of death with odds ratio of 6.7 (95% CI = 1.401–32.198) in a binary logistic regression model with all significant risk factors included.

DISCUSSION

GAS sepsis is a life threatening emergency and our data demonstrate the high case fatality rates among a hospitalized cohort with GAS bacteremia and / or sepsis. Recent surveys among hospitalized and community patients have demonstrated a high incidence and mortality. This rate is substantially higher than the 16% and 19% 7-day case fatality rates observed in the UK (Lamagni *et al.*, 2008) and 11 European countries (Luca-Harari *et al.*, 2009), respectively and reaches rates observed in those with TSS and with the mortality rates in septic shock (Singer *et al.*, 2016). Higher mortality in our series could be due to delayed hospital presentation, underlying patient factors such as debilitation, immune-compromise, overwhelming infection, or more virulent strains of GAS. We have also demonstrated that GAS bacteremia may not always be accompanied by sepsis and that sepsis

syndrome in GAS is an independent predictor of mortality. Recognition of sepsis therefore is a definite priority in patients with possible GAS infection.

In our study, all patients received an appropriate antibiotic in the ED, however, the door-to-antibiotic times were not optimal. Although time to antibiotic administration was longer than that recommended for sepsis (1 hour), the door-to-antibiotic times did not have an effect on survival. We also observed a wide variation in door-to-antibiotic times demonstrating the heterogeneity of GAS presentation making it difficult to diagnose sepsis clinically in these patients (only one patient was identified with a qSOFA sign of low GCS at presentation). These features demonstrate that GAS sepsis is a condition difficult to recognize and control and therefore requires a high index of suspicion in patients presenting with soft tissue infections. Occult GAS bacteremia however, still remains a difficult illness to diagnose. Enhanced diagnostic methods (antigen based point-of-care microfluidic tests or molecular tests) need to be developed to identify patients with GAS bacteremia and sepsis as these may help prevent mortality through earlier aggressive management of such patients.

Once a diagnosis is made, intravenous benzylpenicillin is used as appropriate therapy for GAS bacteremia and sepsis. In our series, we found low compliance with de-escalation of therapy. This points to a need for educating physicians and modifying prescribing behaviours to improve antibiotic stewardship. However, it is important to note that presence of significant comorbidities have an impact on prescribing practices. De-escalation to benzylpenicillin may be detrimental in conditions with potential polymicrobial etiology, e.g diabetic foot and Fournier's, and for sepsis in immunocompromised hosts. Clindamycin was included as part of the regimen in a small number of patients. Observational studies have shown that clindamycin affects survival in GAS infections (Carapetis *et al.*, 2014). Whether this survival effect extends to GAS sepsis is not known and warrants further studies.

Lactate levels >2mmol/L (Singer *et al.*, 2016) should be used among those with soft tissue infection, those over 50 years of age presenting with fever to identify sepsis. The uptake of this test in our series was dismal but seems to be increasing as most values available correspond to data collected over the last 1 year of the study. However, patients with higher lactate identified in the ED still did not survive, even though they were nursed in intensive care. This may be because point-of-care lactate was not used, and laboratory turnaround times may have hindered recognition of severe sepsis. An ED audit of sepsis bundles and lactate uptake and impact in our centre is ongoing.

Seasonal variation was also observed and rates of GAS positive blood cultures increased in winter months. This agrees with the seasonal variation observed in a series of severe GAS infections, as demonstrated by Lamagni *et al.* (2008). Reasons for seasonality of GAS infections and sepsis are poorly understood. Moreover, larger time series analyses will need to be carried out to validate these findings before vaccine evaluations to inform researchers on peak incidence times as these can affect vaccine efficacy measures.

Variation in incidence among age groups is an interesting feature that we have not observed before in other series of GAS infections. Although those over 50 years of age remain the most affected, a concomitant peak in pediatric occurrence with a decrease in infections among those >50 years suggests population- or age-specific *emm* types causing periodic outbreaks.

The data we present here has some notable limitations. We could not comment on incidence of GAS bacteremia as we used a secondary data source to identify cases. Pediatric cases were limited and therefore, our results do not represent the pediatric spectrum of GAS sepsis. Finally, the case series was carried out using data from a single center in an area where a large number of hospitals and laboratories cater to the catchment population and therefore our results cannot be generalized to the entire population.

CONCLUSIONS

We have shown that GAS sepsis has a high mortality and needs to be monitored to establish changes in trends. Earlier identification and aggressive ED treatment is imperative to prevent high mortality and needs to be addressed through regular audits of ED algorithms and bundles.

Authors' statements:
Contributions: SS conceived the study, analysed the data and wrote the manuscript; EK provided the laboratory data and supervised the analysis of laboratory data and trends; FRM assisted with analysis and quality control of data; FM provided clinical details of pediatric patients and edited the manuscript; BJ provided clinical details for adult patients, supervised the analysis, results, and conclusions.

Acknowledgements: None.

Funding: None.

Competing interests: None declared.

Ethical approval: The study was granted ethical clearance by the Ethical Review Committee of the Aga Khan University, Karachi, Pakistan.

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