

Bronchial aspirates glucose level as indicator for MRSA in Intubated mechanically ventilated patients

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ABSTRACT

Objectives: To detect if the level of glucose in bronchial aspirate serves as indicator for the risk of MRSA infection in intubated mechanically ventilated ICU patients.

Methods: 50 critically ill patients was enrolled and were under tight glycemic control to abolish the effect of hyperglycemia on bronchial secretion, if they were expected to require mechanical ventilation for more than 48 hours. Bronchial aspirates were detected for glucose and sent twice weekly for microbiological analysis and whenever an MRSA was expected.

Results: All 50 patients had glucose tested in bronchial aspirates. Glucose was detected in bronchial aspirates of 28 of the 50 patients. Glucose in bronchial aspirates in these patients ranged between (2.9 – 5.1mmol/l). MRSA was detected in 22 patients where 28 were MRSA free of the MRSA patients 19 had positive glucose where glucose was positive in 28 patients of them 19 (86.4%) where MRSA positive to 9 with no MRSA (32.1%). The risk of having MRSA present was markedly increased significantly in the presence of glucose: (p value .001)

Conclusion:

In mechanically ventilated ICU patients, early detection of glucose in their bronchial aspirate improves the ability of the physician to recognize early detection of MRSA this is simple, rapid, inexpensive technique serve as marker for nosocomial MRSA infection

INTRODUCTION

Although *S. aureus* has been causing infections (staph infections) probably as long as the human race has existed, MRSA has a relatively short history, was first noted in 1961, about two years after the antibiotic methicillin was initially used to treat *S. aureus* and other infectious bacteria. The resistance to methicillin was due to a penicillin-binding protein coded for by a mobile genetic element termed the methicillin-resistant gene (*mecA*). In recent years, the gene has continued to evolve so that many MRSA strains are currently resistant to several different antibiotics and is sometimes termed a superbug because of its ability to be resistant to several antibiotics Kock R, et al; 2010

This organism is known for causing skin infections in addition to many other types of infections. There are other designations in the scientific literature for these bacteria according to where the bacteria are acquired by patients, such as community-acquired MRSA (also termed CA-MRSA or CMRSA), hospital-acquired or health-care-acquired MRSA (also termed HA-MRSA or

HMRSA), or epidemic MRSA (EMRSA). Data supplied by the CDC in 2011 suggest this number has declined by about 54% from 2005 to 2011, in part, because of prevention practices at hospitals and home care. In addition, hospital deaths from MRSA infection have declined by about 9,000 per year from 2005-2011. However, the CDC recently estimated about 80,000 infections with 11,000 deaths occurred in 2011, but they suggest that a far greater number of minor infections occurred in both the community and in hospitals Seaman A, 2013.

More than 70% of *S aureus* isolated in ICUs is MRSA Many patients not infected on admission may actually become colonized with MRSA while in the ICU Hill DM1, et al ;2013. Analysis of a large surgical ICU cohort showed that 8% of patients were colonized with MRSA at the time of admission, and that these subjects can serve as a reservoir for the spread of this pathogen. Beyond the hospital, traditional nosocomial strains of MRSA are increasingly implicated as the cause of "healthcare-associated" infections Robotham JV, et al. 2011

The bacterial epidemiology of VAP depends on a panel of factors including mechanical ventilation duration, length of hospital and ICU stays, previous exposure to antimicrobials, local epidemiology and potential epidemic phenomenon in a given ICU. Rapid changes in the oropharyngeal flora of intubated patients (even in the absence of antibiotic exposure) represent a key determinant Hill DM1, et al 2013. as micro aspirations of pharyngeal secretions constitute the leading physiopathological mechanism of VAP. Early-onset VAP (within the first 4 days of mechanical ventilation) Jones RN 2010. However, MDR pathogens may be isolated in early-onset VAP when risk factors exist prior to ICU admission, and even when such risk factors are lacking [notably for *Pseudomonas aeruginosa* and methicillin-resistant [notably for *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) Tacconelli E1, et al 2009 May enlightening the limits of this classification in the current context of bacterial resistance Hyllienmark P, et al 2012.

Philips BJ, et al. 2003 reported that glucose was detected in bronchial aspirate of critically ill patients with stress hyperglycemia when the blood glucose concentrations had increased to (>6.7–9.7 mmol/l).

It was suggested that, increased glucose concentrations in the airways can increase the risk of chest infections, either by providing added nutrition for bacteria or by interfering with normal immune functions Philip BJ, et al (2005)

Van den Berghe G, et al, 2001 found on cardiothoracic intensive care unit (ICU), mortality was decreased by control of blood glucose to normal limits (4.4–6.1 mmol/l) also). This was principally through a reduction in episodes of sepsis, but no mechanisms for this effect were suggested. It has been previously shown that normal airways secretions contain less than 0.5mmol/l glucose (lower limit of standard methods of detection). Wood DM, et al (2004)

The aim of this study to detect the relation between the presences of glucose in bronchial secretion of intubated mechanically ventilated patients and MRSA infection

SUBECTS AND METHODS

Fifty critically ill patients requiring intubation and ventilation were chosen from the New and Old Jeddah Clinic Hospitals. With age ranging between 30 and 80 years old) Patients were included if they were expected to require intubation for more than 48 hours. Patients were excluded if they had had a previous ICU admission during this hospital admission in the period March 2013 to March 2014.

Nineteen patients were admitted for respiratory reasons, five for cardiology reasons, and five for gastroenterology reasons, one for hematological problems, and 15 for neurological reasons, five for infectious reasons, and four for general medicine problems. Severity of illness was measured using the sequential organ failure assessment (SOFA) system. Outcome was assessed at discharge from the ICU and after 6 months

All patients were subjected to the following:

Detailed history and thorough medical examination;

Chest x-ray, CBC, Blood glucose and were under tight glycemic control aiming at blood glucose level between 90-144 mg/dl as described by Van den Berghe G, et al 2001.

Bronchial aspirate analysis were collected by a bronchial aspirate with fiber optic bronchoscopy using a large channel bronchoscopy (Olympus BF 1T-20D; Olympus, New Hyde Park,NK,USA). The fiberoptic bronchoscopy (FOB) was inserted through the endotracheal tube via a steri connector (Carden Swivel Connector, Bivona, Griffith Microscience Inc, IN,USA and analyzed for glucose and sent twice weekly for microbiological analysis and whenever an infection was suspected. Glucose was measured simultaneously in arterial blood using a near patient glucose analyzer (vitros 250, Johnson and Johnson co). Bronchial aspirates were analyzed for glucose at the bedside using a glucose oxidase stick, followed by more precise glucose analysis of bronchial aspirate filtrate and bronchial aspirates (Glucose appeared in bronchial aspirates as blood glucose levels exceeded 6.7–9.7 mmol/l).

Microbiological methods:

Samples for microbiological evaluation were collected in sterile receptacles. Sample quality was evaluated according to the criteria of Murray PR and Washington JA 1975. and samples were immediately seeded on reaching the laboratory. *S. aureus* colony counts of >103 cfu/mL were considered to be significant. Quantitative culture was performed on arrival of the samples at the laboratory. *S. aureus* at >105 cfu/mL was considered significant. Somerville GA, *et al* (2002).

The statistical analysis:

Data were statistically described in terms of mean \pm standard deviation (\pm SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing normally distributed data and Mann Whitney *U* test for independent samples when data not normally distributed. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows

RESULTS

Of the 50 patients, 32 were men and 18 women. All 50 patients had glucose tested in bronchial aspirates. Glucose was detected in bronchial aspirates of 28 of the 50 patients. Glucose in bronchial aspirates in these patients ranged between (2.9 – 5.1mmol/l). MRSA was detected in 22 patients where 28 were MRSA free of the MRSA patients 19 had positive glucose where glucose was positive in 28 patients of them 19 (86.4%) where MRSA positive to 9 with no MRSA (32.1%). The risk of having MRSA present was markedly increased significantly in the presence of glucose: (p value .001) table (1).figure (1) There were no significant correlation between incidence of MRSA and length of stay in ICU (p value 0.015) (table1), age of the patient (p value 0.969) (table1), diabetes mellitus (p value 0.254) (table 1), chest infiltrates (p value 0.302) (table 1), or leucocytic count (p value 0.080) (table 1). There was no correlation between age of the patient and blood glucose level in bronchi (p value 0.740). (table2).figure (2)

Table 1: Demographic, clinical and lab parameters between the study groups

Item	MRSA (n = 22)	No MRSA (n = 28)	P value
Age (mean ± SD, years)	57.0 ± 16.3	58.9 ± 9.4	0.969
DM [count (%)]	16 (72.7%)	16 (57.1%)	0.254
TLC (mean ± SD, x1000/cmm)	11.3 ± 5.3	16.3 ± 9.8	0.080
Glucose in bronchial lavage [count (%)]	19 (86.4%)	9 (32.1%)	< 0.001
CXR infiltration [count (%)]	6 (27.3%)	4 (14.3%)	0.302
ICU stay (mean ± SD, days)	20.7 ± 18.2	9.04 ± 7.3	0.015

Table 2: Comparison of age (years) between cases with glucose in bronchial lavage and those with no glucose in bronchial lavage

Item	Glucose in bronchial lavage (n = 28)	No glucose in bronchial lavage (n = 22)	P value
Age (mean ± SD, years)	57.5 ± 13.4	58.7 ± 12.2	0.740

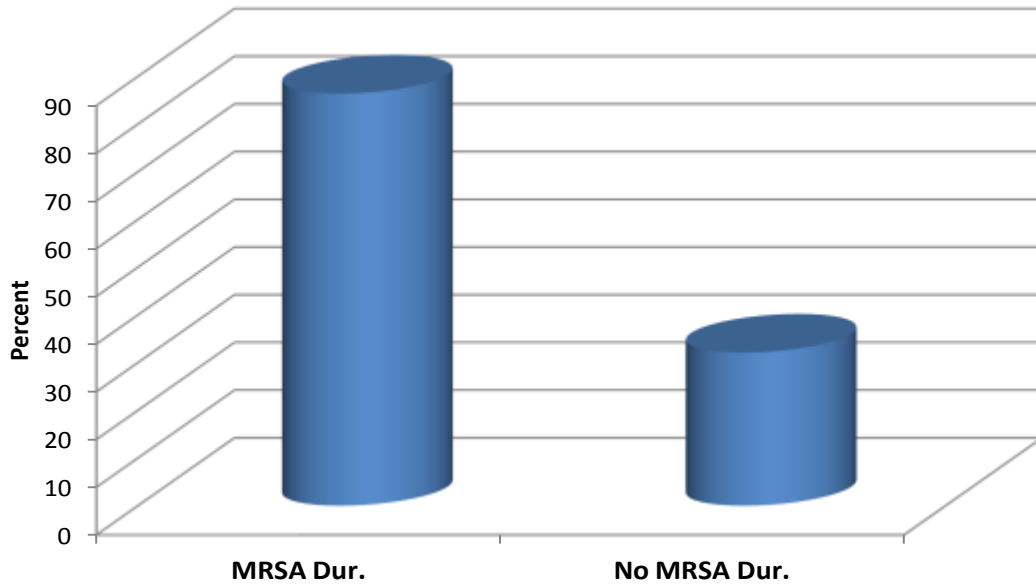


Figure 1 Percentage of presence of glucose in bronchial lavage between cases with MRSA during ICU stay and those without MRSA during ICU stay

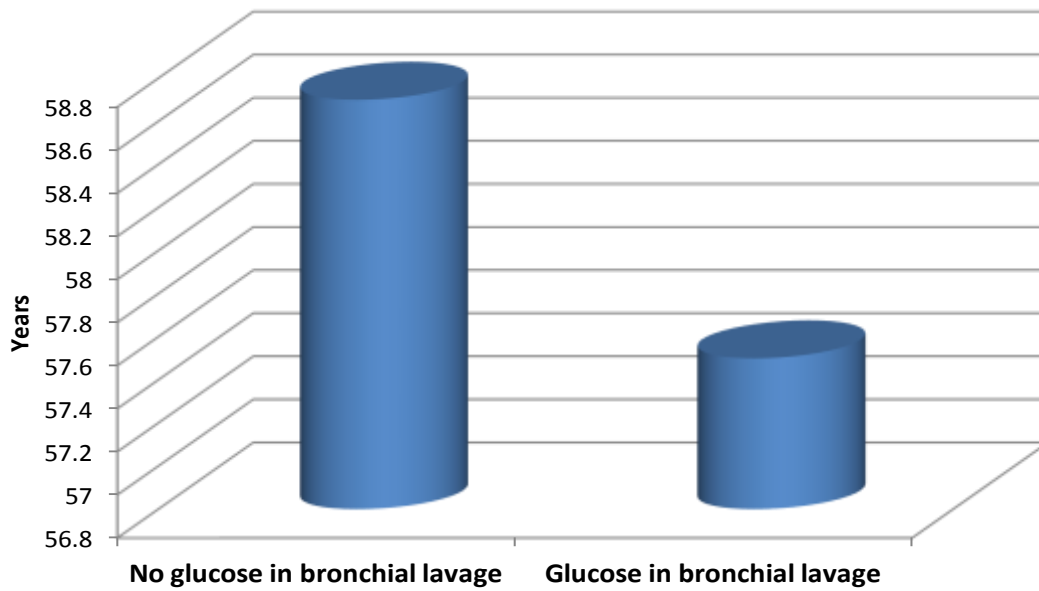
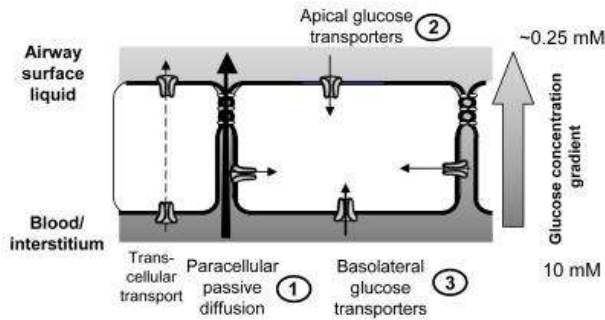
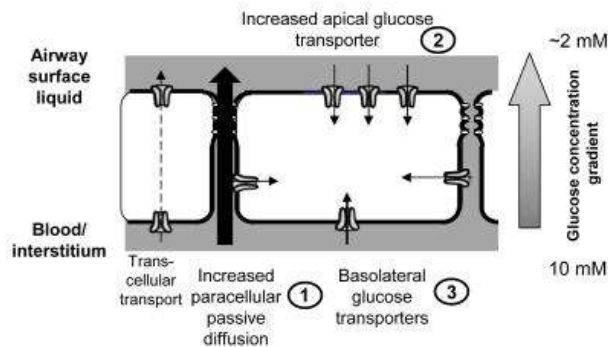


Figure 2 Mean age (years) between cases with glucose in bronchial lavage and those without glucose in bronchial lavage

Normal airway



Airway treated with pro-inflammatory mediators (72 hours)



Proposed mechanisms controlling glucose concentrations in airway surface liquid

Normal airway: Under normal conditions, glucose predominantly diffuses from blood/interstitial fluid across the respiratory epithelium into airway surface liquid (ASL) via paracellular pathways and this is limited by the permeability of the epithelium. Apical and basolateral facilitative GLUT-mediated transport restricts glucose accumulation in ASL (there is little evidence for Na^+ -glucose co-transport in these airway cells). Glucose taken up into the cell is rapidly metabolised. This is critical to maintain low intracellular glucose concentrations which provide a driving force for glucose uptake via GLUT transporters. It also limits the transcellular transport of glucose and predicts that ASL glucose concentrations equilibrate with intracellular glucose concentration. **Airway treated with pro-inflammatory mediators:** Pro-inflammatory mediators reduce transepithelial resistance and increase paracellular diffusion of glucose across the epithelium into ASL. Whilst apical GLUT-mediated glucose transport and GLUT2 and 10 abundance is increased, this is insufficient to prevent a rise in glucose concentration in the ASL.

DISCUSSION:

MRSA pneumonia as with other infections caused by this organism is usually of nosocomial origin Robotham JV, et al 2011 ; and it was suggested that staph aureus preferentially use glucose during the exponential phase of growth Somerville GA, *et al* (2002) glucose also affect the virulence of bacteria, Spomenk L, et al 2004. Our present study demonstrated the relation between the presence of glucose in the bronchial secretion and the development of MRSA infection. The factors associated with MRSA pneumonia are advanced age, prolonged hospitalization, gravity of the underlying diseases, especially lung diseases prior antibiotic therapy, and surgery or other invasive maneuvers (vesicular probe, central catheters, and endotracheal intubation) Kock R, et al 2010 . There were no differences in clinical findings, radiological in MRSA positive and MRSA negative in our study and this in agreement with a study published in 1994, Iwahara et al. 1994. analyzed 32 patients with MRSA pneumonia and found these same risk factors for the acquisition of the infection, with the exception of the lung diseases.

Geerlings SE, et al, (2002) suggested that glucose may virulence the bacteria. As glucose is known to be important element in formation of biofilm in staph aureus protecting them from adverse environments, thus keeping bacteria pathogenicity by preventing clearance phase from host in many numbers of infections of them pneumonia. Where the presence of glucose in the airway may interfere with local immune process as virus replicate more rapidly in the lung of animal models and decrease the respiratory burst of alveolar macrophages impairing killing of bacteria this was interpreted by blocking the carbohydraste binding domains of proteins needed for recognition of attaching pathogens, which might explain the increase incidence of MRSA where glucose concentration in bronchial secretion was higher in our study group figure (1).Kalsi KK and Baker EH 2008. On the other hand concluded that systemic hyperglycemia in animals models produce pro inflammatory cytokines including TNK and IL6 which in turn induce insulin resistance causing structural changes to animal lung .Although it was shown previously that hyperglycemia causes the appearance of glucose in the airway secretions and in those studies significant hyperglycemia were observed in patients with glucose in their bronchial secretion. However there were patients with glucose in bronchial secretion with normal or even low blood glucose levels at time of testing in our study hyperglycemia was not targeted Popovich KJ, et al 2013& Cai D, *et al* (2005).In humans the air way surface liquid glucose (ASL) concentration are elevated when the airway epithelium is inflamed or when blood glucose is elevated. Nasal glucose concentration are undetectable (less than1mM) in healthy volunteers but detectable in 50% in patients with symptoms of viral rhinitis (~1mM)and in 90% with diabetes mellitus (~4mM) Breath glucose is elevated in people with lung inflammation due to cystic fibrosis (2+-1.1mM), with hyperglycemia due to diabetes mellitus (1.2+-0.07mM) and to highest in people with both cystic fibrosis and diabetes mellitus (4.0+-2.0mM) Baker EH, et al 2007. . In intensive care patients with elevated airway surface liquid glucose concentration are more likely to have respiratory infection, particularly with methicillin-resistant staphylococcus aureus, than those with normal ASL glucose and this was the result in our study group. Number of possible explanation could explain the presence of glucose in airway secretion as it may enter the airway through leaking inflamed tissue. Fagon JY 2011.

Philip et al 2005 noted that volunteers with common cold glucose appear in their nasal secretion while they have normal blood level and disappear once symptom resolved. Another explanation

is possible impairment of glucose clearance in critically ill patient Popovich KJ, et al 2013 & Baker EH, et al 2007 where baker et, al 2007 found low level of glucose in airway secretion against the concentration gradient by active transport. Nathani N,et al 2006 studied EBC glucose was higher in non-survivors compared to survivors. EBC glucose from infected patients was also higher than non-infected ones despite the presence of statistical significant difference between their serum glucose. They concluded that EBC glucose is potential marker of both infection/outcome in ARDS. They suggested that EBC collection might prove to be useful tool in guiding treatment and intervention in ARDS Nathani N,et al 2006.

Despite these findings, the role of glucose in lung inflammation remains uncertain. Diabetes mellitus was found to be a negative predictor for acute respiratory distress syndrome (ARDS) secondary to sepsis. Although, when hyperglycemia was used as the predictor rather than a history of diabetes mellitus and the data were adjusted for confounding variables, there was no difference in the incidence of ARDS between groups Moss M, et al 2000 & Wood DM, *et al*(2004), hypothesized that increased glucose concentrations in the airways increased the risk of chest infections, either by providing added nutrition for bacteria or by interfering with normal immune functions

As MRSA follow the detection of glucose in bronchial secretion in most cases, suggesting that glucose could have caused or promoted MRSA growth Gordon RJ and Lowly FD 2008 which also was detected in our study group ,although we were unable to quantitative the MRSA load in individual patients and thereby differentiate between colonization and infection. It was suggested that staph aureus preferentially use glucose during the exponential phase of growth. Indeed, exit from this phase is in part determined by a lack of easily catabolisable carbohydrates Somerville GA *et al* (2002).

James P. Garnett, et al 2012 confirmed that the exposure of airway epithelium cell mono layers to pro inflammatory mediators increase ASL glucose concentration and was shown that the underlying mechanism is increased basolateral to apical paracellular glucose flux, which exceed compensatory up regulation of apical glucose transport. Because elevated ASL glucose concentration compromise the sterility of the lung and increase susceptibility to infection, insight into disruption of airway glucose homeostasis under inflammatory conditions could provide new therapeutic targets for lung infection Pezzulo AA, et al 2011 Our study has documented a direct relation positive correlation between the presence of glucose in bronchial secretion of the critically ill patients and staph aureus infections, particularly MRSA. These results in agreement with Philip BJ, et al (2005) who detected the glucose in bronchial secretion of 58 of 98 patients and those patients were more likely to have pathogenic bacteria than patients without glucose detected in bronchial secretions.

Conclusion:

This simple , inexpensive and rapid technique in evaluating the bronchial secretion for the glucose might be opportunity to detect MRSA in incubated mechanically ventilated patients earlier so improve the clinicians diagnosis , patient hospital stay days and costs.

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