



Short Communication

Isolates of *Staphylococcus aureus* Induce Selected Metabolites in Human Polymorphonuclear Leucocytes

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ABSTRACT

The study was aimed at determining levels of reactive oxygen intermediate (ROI) and reactive nitrogen intermediate (RNI) in human polymorphonuclear leukocytes induced by different *Staphylococcus aureus* isolates, and correlating them with antibiotic resistance. For this, one hundred different isolates of *S. aureus* were obtained from various hospitals of Lahore and screened for methicillin resistance. Polymorphonuclear leucocytes, collected from the blood of healthy individuals, were exposed to bacterial strains at 37°C in the presence of opsonin. Human polymorphonuclear leucocytes phagocytosed methicillin sensitive *S. aureus* (MSSA) more than that compared to methicillin resistant *S. aureus* (MRSA). However, MRSA produced more superoxide and nitric oxide as compared to MSSA.

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Authors' Contribution

NA designed the experiments. NJ and RT performed experimental work. ZA analyzed the data and wrote the article.

Key words

S. aureus, Polymorphonuclear leucocytes, Nitric oxide, Superoxide.

Staphylococcus aureus is one of the important Gram positive pathogens that are associated with fetal community and nosocomial diseases like abscesses, septicemia, septic arthritis and infective endocarditis. Staphylococcal infections are caused by methicillin sensitive *Staphylococcus aureus* strains (MSSA). Currently all over the world, resistance of a number of antibiotics is being observed against *S. aureus* infections (Javed *et al.*, 2011). In addition to resistance against methicillin and β -lactam, *S. aureus* is also exhibiting resistance to vancomycin, linezolid, and daptomycin, which are considered to be the drugs of last resort to treat these infections (Hirschwerk *et al.*, 2006).

Innate immune system contains a highly conserved strategy against a wide range of bacterial, fungal, protozoal and viral pathogens (Janeway and Medzhitov, 2002). Neutrophils or polymorphonuclear leucocytes (PMNLs) belong to the class of professional phagocytes, which is most plentiful in blood and it usually arrives early at the site of inflammation. Phagocytosis of microorganisms by neutrophils is an important component in the host defense against bacterial infections (Silverstein and Steinberg, 1990). The process of microbial killing in which oxygen is an integral part causes production of reactive oxygen species (ROS) like superoxide, which are microbicidal (Roos *et al.*, 2003). Nitric oxide (NO) is one of the main reactive nitrogen

intermediate (RNI) which is generated from arginine metabolism and it plays a critical role in the functions of macrophages, like cytotoxicity toward cancerous cells and microbes (Stuehr *et al.*, 1991). According to latest research, NO performs an important role in infection, wound healing, vasodilatation and angiogenesis. It is apparent that both RNI and ROS are involved in killing of bacteria. It is possible that different isolates of *S. aureus* based upon their antibiotic phenotype may vary in their ability to generate these metabolites and therefore, the degree of induction of these metabolites is related to antibiotic resistance. To the best of our knowledge, this is the first report about the ability of *S. aureus* isolates based on their antibiotic phenotype to induce metabolites in human polymorphonuclear leucocytes. However, Wilson *et al.* (1996) has described that calcium induces transient increase on intracellular killing of *S. aureus* by regulating toxic oxygen metabolites in the phagosomes of human neutrophils. In this study, we examined the abilities of different isolates of *S. aureus* to induce the production of NO and superoxide radicals in human PMNLs.

Materials and methods

Isolation and PMNL and their interaction with *S. aureus* isolates

Thirty five methicillin susceptible *S. aureus* (MSSA) and forty methicillin resistant *S. aureus* (MRSA) from different public and private hospitals of Lahore were phenotypically and biochemically characterized.

PMNL were isolated from healthy male individuals

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of 22-25 years age who did not have history of pyogenic infection during the last six months; by dextran (T-500) density gradient centrifugation (Javed *et al.*, 2009). Freshly isolated PMNLs and overnight incubated culture of both MSSA and MRSA were co-cultured and allowed to interact in 1:50 ratio by opsonin dependent phagocytosis at 37°C (Javed *et al.*, 2009).

The concentration of superoxide was determined by reduction of ferricytochrome c which was superoxide dismutase (SoD) inhabitable. The isolated PMNLs were suspended in Hank's buffered salt solution (HBSS) containing 100µM ferricytochrome c. Afterwards, 200µL of cell suspension was added to 96 well microtitre plate. It was pre-warmed at 37°C for 5 min before stimulation. In control well, 12.5 µg/mL of SOD (Sigma, St. Louis) was added. The cells were stimulated with 5µL of opsonized bacterial suspension. The change in absorbance at 550 nm was recorded for 10 min with two measurements per minutes at 37°C by microplate reader which had inbuilt plate shaker facility (Bio-Rad Model: 468). Superoxide production was calculated by an absorption coefficient of 21 mM⁻¹ cm⁻¹ for cytochrome c after subtracting the background values (Rada *et al.*, 2004).

The amount of NO was determined in the supernatant without cells by using Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2% orthophosphoric acid) (Sigma, St. Louis). The supernatant was mixed with equal volume of Griess reagent and incubated at room temperature for 10 min after which absorbance was measured at 550 nm using ELISA plate reader (Bio-Rad Model 468). A standard curve of sodium nitrite (Sigma, St. Louis) was used to establish NO concentration in the sample, (Bekker *et al.*, 2001).

The mean and standard deviation of the quantitative variables such as amount of superoxide and nitric oxide was evaluated using Microsoft Excel 2010. Graphs were prepared using Graphpad Prism 5.

Results and discussion

Figure 1 shows concentration of superoxide and nitric oxide (NO) produced by PMNLs after interaction with MRSA and MSSA. The range of superoxide and NO production by PMNLs (1 × 10⁶) for MRSA group was 40 - 85 µmol with a mean ± SD of 55.05 ± 10.23 µmol and 26-54 µmol with a mean ± SD of 42.1 ± 7.3 µmol while for MSSA group it was 24 - 41 µmol with a mean ± SD of 32.29 ± 4.84 µmol for superoxide 27-39 µmol with a mean ± SD of 31.2 ± 3.6 µmol for NO. There were statistically significant difference between MRSA and MSSA groups in production of superoxide and NO, respectively by PMNLs (p < 0.001).

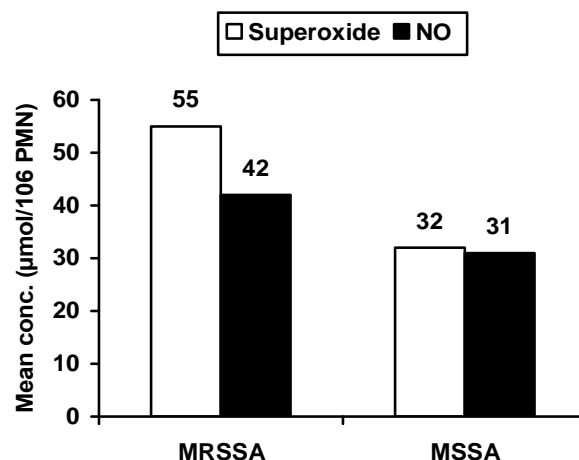


Fig. 1. Superoxide and nitric oxide (NO) produced by PMNs by MRSA and MSSA.

Nilsson-Augustinsson *et al.* (2004) reported that *S. aureus* gives rise to significant oxidative response within PMNLs compared to other Staphylococci organism. During phagocytosis, respiratory burst occurs in PMNLs which causes production of various bactericidal metabolites within the cell (Hampton *et al.*, 1996). Superoxide radicals are among these reactive oxygen metabolites which are produced during respiratory burst in PMNLs. During phagocytosis, in addition to ROI, PMNLs also produce other metabolites such as RNI and NO. NO has been reported as one of the potent bactericidal agents but according to Goode and colleagues. (Goode *et al.*, 1994) the importance of NO in neutrophil mediated host defense is not significant because NO is produced in small amount by human PMNLs. It has been reported that NO mediated killing is a delayed event but NO has a pivotal role in human PMNL's killing of Staphylococci under compromised immunity (Kaplan *et al.*, 1996).

Production of superoxide by MRSA and MSSA groups was positively correlated with their ability to produce NO, showing the values of p < 0.001 and r = 0.584 (Fig.2). It means, whenever there is increased production of superoxide there will be amplified production of NO and *vice versa*. Conversely, there was no significant correlation between the production of superoxide and NO by MRSA and MSSA because p-value were <0.869 and <0.144, while r values were 0.027 and 0.252, respectively. Hence, the present study provides a new comparative approach between the abilities of MRSA and MSSA to produce ROI and RNI by human PMNLs.

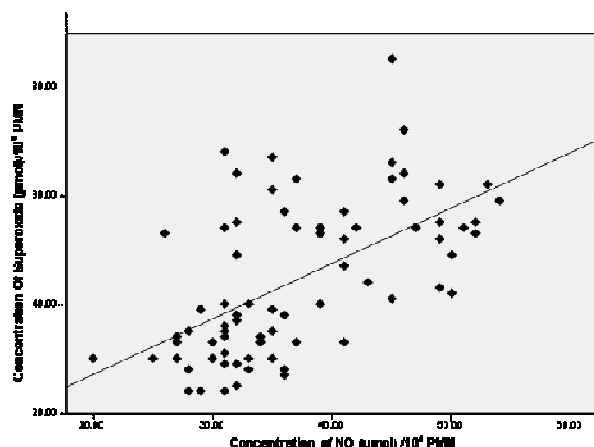


Fig. 2. Correlation between the concentration of superoxide and nitric oxide production

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Statement of conflict of interest

Authors have declared no conflict of interest.

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