METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN ANIMALS

Bhandari B. B. and Jhala M. K.
College of Veterinary Science and Animal Husbandry, Anand Agricultural University
Anand – 388 001, Gujarat, India

ABSTRACT
In recent years, Methicillin-resistant Staphylococcus aureus (MRSA) has been increasingly reported as emerging problem in veterinary medicine, particularly in small animals. Strains isolated from pet animal cases were usually indistinguishable from those isolated from human. Pets become infected through contact with infected people, and those pets in turn pass MRSA back to humans indicating its zoonotic importance. MRSA are not only carried by pet animals but can also cause clinical disease in animals. MRSA have been found in number of animals including Dogs, Cats, Horses, Sheep, Pigs and Chickens. Methicillin was first introduced in human medicine in 1959, when it was used for treating penicillin-resistant staphylococcal infections. Methicillin is classified under narrow spectrum β-lactamase resistant penicillins. It acts by interfering primarily with the synthesis of bacterial cell wall by binding to penicillin binding proteins. Methicillin resistance is mediated by the mecA gene encoding for penicillin-binding protein 2a (PBP-2a). mecA gene is located on a mobile genetic element called the staphylococcal cassette chromosome (SCCmec). Expression of PBP-2a is controlled by mecR1 and mecI regulator genes located on mecA gene. Mutations in the mec regulators may result in expression of PBP2a and the strain becomes highly resistant to Methicillin. MRSA typing systems are important for investigation of outbreaks to aid the clinical treatment of patient, discrimination between successive and recurrent infections and understanding epidemiology of the infections. Molecular typing methods are the most important which mainly includes, Chromosomal DNA analysis after REA, Pulsed field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), SCCmec typing and spa typing. Regarding public health significance, several reports are suggesting that animals may serve as reservoirs for MRSA infection to humans. Control of MRSA in animals includes, preventing introduction of infection at veterinary hospitals, preventing transmission from animal to human and human to animal by taking hand hygiene and related measures, preventing transmission from animal to animal by isolating all suspect cases and preventing indirect transmission following high standards of cleaning and disinfection.

KEYWORDS: MRSA, animals, zoonotic.

1. INTRODUCTION
Methicillin-resistant Staphylococcus aureus (MRSA) is emerging as a zoonotic and veterinary bacterial pathogen of public health importance. It causes nosocomial and community onset infections. MRSA is considered being resistant to virtually all available beta-lactam antibiotics which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin and cephalosporins). In last four decades, it has shown increasing endemic and epidemic spread (Kuehnert et al., 2005). MRSA associated infections in human patients impose serious burden in terms of treatment costs and cause significant morbidity and mortality (Bratu et al., 2005). MRSA patients stay in hospital longer with higher cost of treatment than do patients with non resistant staphylococcal infections (Cosgrove, 2006). MRSA infections accounted for an estimated 19,000 human deaths in U.S. in 2005, more than AIDS. From 1999 to 2005, Hospital treated MRSA infections have more than doubled i.e. from approximately 127,000 in 1999 to 278,000 in 2005 (Klein et al., 2007).

Veterinary significance
In recent years, MRSA has been increasingly reported as emerging problem in veterinary medicine, particularly in small animals and equine practices. Strains isolated from pet animal cases usually indistinguishable from those isolated from human. Pets become infected through contact with infected people, and those pets in turn pass MRSA back to humans indicating its zoonotic importance. MRSA are not only carried by pet animals but can also cause clinical disease in animals. MRSA have been found in number of animals including Dogs, Cats, Horses, Sheep, Pigs and Chickens. Methicillin was first introduced in human medicine in 1959, when it was used for treating penicillin-resistant staphylococcal infections. Methicillin is classified under narrow spectrum β-lactamase resistant penicillins. It acts by interfering primarily with the synthesis of bacterial cell wall by binding to penicillin binding proteins. Methicillin resistance is mediated by the mecA gene encoding for penicillin-binding protein 2a (PBP-2a). mecA gene is located on a mobile genetic element called the staphylococcal cassette chromosome (SCCmec). Expression of PBP-2a is controlled by mecR1 and mecI regulator genes located on mecA gene. Mutations in the mec regulators may result in expression of PBP2a and the strain becomes highly resistant to Methicillin. MRSA typing systems are important for investigation of outbreaks to aid the clinical treatment of patient, discrimination between successive and recurrent infections and understanding epidemiology of the infections. Molecular typing methods are the most important which mainly includes, Chromosomal DNA analysis after REA, Pulsed field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), SCCmec typing and spa typing. Regarding public health significance, several reports are suggesting that animals may serve as reservoirs for MRSA infection to humans. Control of MRSA in animals includes, preventing introduction of infection at veterinary hospitals, preventing transmission from animal to human and human to animal by taking hand hygiene and related measures, preventing transmission from animal to animal by isolating all suspect cases and preventing indirect transmission following high standards of cleaning and disinfection.
MRSA in animals

found in clinically affected dogs and cats respectively (Walther et al., 2008). 16% Pigs 16% positive on nasal swabs, 7.4% positive on both nasal and rectal swabs and 1.4% positive on rectal swabs only (Khanna et al., 2008).

Twelve of 487 dogs (2.5%), six of 48 cats (12.5%), 5 of 12 horses (42%), and 1 of 2 pigs samples were detected positive for MRSA (Lin et al., 2010). (Table 1)

### TABLE 1. Summarized chronology of publications reporting MRSA infections in animals

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Authors</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Devriese et al. (1972)</td>
<td>Isolation of MRSA from cows with mastitis</td>
</tr>
<tr>
<td>2</td>
<td>Devriese and Hommez, (1975)</td>
<td>Isolations of MRSA from dairy cows, suggested to be of human origin</td>
</tr>
<tr>
<td>3</td>
<td>Scott et al. (1988)</td>
<td>Cat suspected to be a source of MRSA outbreak in geriatric ward</td>
</tr>
<tr>
<td>4</td>
<td>Smith et al. (1989)</td>
<td>Isolation of coagulase-positive Staphylococcus sp. (12% of isolates were oxacillin resistant) from orthopedic implant sites in dogs</td>
</tr>
<tr>
<td>5</td>
<td>Cefai et al. (1994)</td>
<td>Repeated nasal carriage of MRSA in two nurses linked to pet dog</td>
</tr>
<tr>
<td>6</td>
<td>Anzai et al. (1996)</td>
<td>Isolation of MRSA from 13 mares with metritis and a stallion with a skin lesion</td>
</tr>
<tr>
<td>7</td>
<td>Hartmann et al. (1997).</td>
<td>MRSA isolated from leg wound of horse</td>
</tr>
<tr>
<td>8</td>
<td>Shimizu et al. (1997).</td>
<td>PGFE typing of equine MRSA isolates revealed pattern distinct from human isolates</td>
</tr>
<tr>
<td>9</td>
<td>Lilenbaum et al. (1998)</td>
<td>Isolation of MRSA from skin scrapings from clinically normal cats</td>
</tr>
<tr>
<td>10</td>
<td>Seguin et al. (1999)</td>
<td>MRSA outbreak in equine patients attending a veterinary hospital. Closely related MRSA isolates also obtained from hospital personnel</td>
</tr>
<tr>
<td>11</td>
<td>Pak et al. (1999)</td>
<td>Isolation of MRSA from 12 dogs hospitalized due to a variety of clinical conditions</td>
</tr>
<tr>
<td>12</td>
<td>Tomlin et al. (1999)</td>
<td>MRSA infection in 11 dogs associated with surgery, traumatic wounds or recurrent pyoderma</td>
</tr>
<tr>
<td>13</td>
<td>Gortel et al. (1999)</td>
<td>MRSA isolates from wounds and skin lesions in dogs</td>
</tr>
<tr>
<td>14</td>
<td>Lee (2003).</td>
<td>MRSA isolated from dairy cows (milk samples) and chickens (muscle or joint samples)</td>
</tr>
<tr>
<td>15</td>
<td>Manian (2003)</td>
<td>Identical PGFE type of MRSA isolated from pet dog of owners with recurrent MRSA infection</td>
</tr>
<tr>
<td>16</td>
<td>Goni et al. (2004).</td>
<td>MRSA isolated from case of ovine mastitis</td>
</tr>
<tr>
<td>17</td>
<td>Van Duijkeren et al. (2004)</td>
<td>MRSA isolated from two dogs with wounds</td>
</tr>
<tr>
<td>18</td>
<td>Rich and Roberts (2004)</td>
<td>MRSA isolates (n=95) reported from dogs (69), cats (24), a horse and a rabbit. Most isolates obtained from wound infections, postoperative infections or from the skin</td>
</tr>
<tr>
<td>19</td>
<td>Boag et al. (2004)</td>
<td>MRSA isolates (n=12) from dogs (7) and cats (5)</td>
</tr>
<tr>
<td>20</td>
<td>Voss et al. (2005)</td>
<td>Association between pig farming and high MRSA carriage rates</td>
</tr>
<tr>
<td>21</td>
<td>Bender et al. (2005)</td>
<td>Isolation of MRSA from non-healing abscess in a cat</td>
</tr>
<tr>
<td>22</td>
<td>O’Mahony et al. (2005)</td>
<td>MRSA isolated from dogs (14), horses (8), a cat, a rabbit and a seal. Isolates were also obtained from attendant veterinary personnel. Non-equine MRSA isolates had PGFE pattern indistinguishable from most prevalent MRSA strain in human population. Distinct PGFE pattern for equine MRSA isolates</td>
</tr>
<tr>
<td>23</td>
<td>Kwon et al. (2005)</td>
<td>SCC mec characterization of MRSA from bovine milk</td>
</tr>
<tr>
<td>24</td>
<td>Loeffler et al. (2005)</td>
<td>MRSA isolated from staff, dogs and environment of a small animal referral hospital</td>
</tr>
<tr>
<td>25</td>
<td>Middleton et al. (2005)</td>
<td>14% of patients (65) with S. aureus infection at 7 veterinary teaching hospitals infected with MRSA</td>
</tr>
<tr>
<td>26</td>
<td>Hanselmann et al. (2005)</td>
<td>1% nasal MRSA carriage rate in dogs in referral hospitals</td>
</tr>
<tr>
<td>27</td>
<td>Weese et al. (2005)</td>
<td>MRSA isolates from horses and horse personnel typed as SCC mec IV and distinct from epidemic MRSA types predominant in human population</td>
</tr>
<tr>
<td>28</td>
<td>Weese et al. (2005)</td>
<td>4.7% isolation rate of MRSA in screened horses rising to 12% using targeted surveillance</td>
</tr>
<tr>
<td>29</td>
<td>Baptiste et al. (2005)</td>
<td>MRSA isolates from dogs and staff at veterinary hospital identical by PFGE analysis to epidemic MRSA type prevalent in human population. Five distinct MRSA strains isolated from horses</td>
</tr>
<tr>
<td>30</td>
<td>Abbott et al. (2006)</td>
<td>0.6% MRSA carriage in dogs rising to 8% in dogs clinically assessed as suspect cases</td>
</tr>
<tr>
<td>31</td>
<td>Leonard and Markey (2006)</td>
<td>MRSA isolates from postoperative infection sites in five dogs and from nare of veterinary surgeon indistinguishable by PFGE.</td>
</tr>
<tr>
<td>32</td>
<td>Cuyn et al. (2006)</td>
<td>Approx. 0.48% of equine cases presented at veterinary teaching hospital infected with MRSA. Long term nasal carriage of MRSA in two attending veterinarians</td>
</tr>
<tr>
<td>33</td>
<td>Strommeneger et al. (2006)</td>
<td>MRSA isolates from dogs and cats closely resemble hospital-derived isolates in local human population</td>
</tr>
<tr>
<td>34</td>
<td>Weese et al. (2006)</td>
<td>Animal to human and human to animal transmission of MRSA suspected following</td>
</tr>
</tbody>
</table>
3. IDENTIFICATION OF MRSA

S. aureus (including MRSA strains) are clustering, facultative aerobic, Gram-positive cocci with intrinsic ability to ferment carbohydrates, producing white to deep yellow pigmentation on solid culture media. They also ferment mannitol turning manniot salt agar yellow. The organisms produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins used for their identification (Bannerman, 2004).

**Disc diffusion test**
Using Mueller Hinton agar (MHA) plates, MRSA strains exhibit resistance to oxacillin or methicillin (1 or 5µg/disc: zone of inhibition < 14mm in diameter used as marker for all β-lactams).

**Oxacillin MIC test**
Gradient plates of MHA containing 2% NaCl with doubling dilutions from 0.25 µg/ml to 256 µg/ml of oxacillin are prepared. S. aureus inoculum is prepared by diluting 0.5 McFarland equivalent suspension of a strain with sterile normal saline to the concentration of 104 CFU/ml. The plates are spot inoculated and incubated at 35 °C for 24 h. An oxacillin MIC of less than or equal to 2 µg/ml is indicative of susceptible and that of > 2 µg/ml resistant (NCCLS, 2003).

**Detection of meca gene by PCR**
Using meca gene specific primers, MRSA can be detected by PCR methods (Geha et al., 1994).

4. MECHANISM OF ACTION AND RESISTANCE

**Mechanism of action**
Methicillin is classified under narrow spectrum beta lactamase resistant penicillins. It acts by interfering primarily with the synthesis of bacterial cell wall and produce effect by binding to penicillin binding proteins (PBPs). PBPs essentially involved in the maintenance of normal cell morphology and viability of bacteria. PBPs are grouped in to six types according to their molecular weight. Drugs occupy the active site of transpeptidase enzyme (PBP) and inactivate it. Inactivation of transpeptidation in cell wall synthesis leads to blockage of cell wall synthesis (Walther et al., 2008).

**Mechanism of resistance**
Mutation on meca gene which results in modification to PBP - 2a, results in drugs not binding to target site and organism becomes resistant to β-lactams and other antibiotics with the same target site. MRSA uses efflux phenomenon resulting in continuous pumping of antimicrobial drugs out of bacterial cell. Alteration in outer-membrane proteins which limit the access of drugs to cell (Stevens, 2004). PBP - mediated resistance in MRSA suggested to take various forms and may arise from overproduction of PBP, acquisition of a foreign PBP with low affinity, recombination of susceptible PBP with more resistant varieties, point mutations within PBPs that consequently lower their affinity for β-lactams (Deurenberg et al., 2007).

Methicillin resistance requires the presence of meca gene. This meca gene is chromosomally located on a mobile genetic element called the staphylococcal cassette chromosome (SCC). Further meca gene encodes for penicillin-binding protein 2a (PBP-2a) and responsible for synthesis of penicillin-binding protein 2a (PBP2a; also called PBP2) a 78-kDa protein. Expression of PBP-2a is controlled by mecr1 and mecr1 regulator genes located upstream of meca gene. A mutation in the mecr regulators leads to expression of meca gene (PBP-2a). A PBP2a substitute for the other PBPs and, because of its low affinity for all β-lactam antibiotics, enables staphylococci to survive exposure to high concentrations of these agents (Figure 1). Thus, resistance to methicillin confers resistance to all β-lactam agents, including cephalosporins (Deurenberg et al., 2007).

5. BASED ON SCCmec TYPE, MRSA CLASSIFIED IN FIVE GROUPS

The 2.1-kb meca gene is located on SCCmec. Currently, five main types of SCCmec (types I–V) have been distinguished, ranging in size from 20.9 to 66.9 kb. SCCmec types I (34.3 kb), IV (20.9–24.3 kb) and V (28 kb) encode exclusively for resistance to β-lactam antibiotics. In contrast, SCCmec types II (53.0 kb) and III (66.9 kb) determine multi resistance, as these cassettes contain additional drug resistance genes on integrated plasmids (pUB110, p258 and pT181) and a transposon (Tn554) (Deurenberg et al., 2007).

Classification of MRSA strains based on SCCmec gives epidemiological features of strains. That is, possible sources of acquisition and dissemination (nosocomial versus community), nature of drug-resistance (single versus multiple), genome characteristics viz: size of genome. Type of ribosome found in individual strain type. Type IV MRSA strains originating from community (Ca-MRSA) found to possess higher prevalence of certain virulence factors as compared with non methicillin -
resistant *S. aureus* (NMRSA) and health care associated MRSA (HA - MRSA) (Akande, 2010). CA- MRSA believed to have inherent potential for severe disease than NMRSA, and broader antibiotic resistance than typical HA – MRSA (Said – Salim *et al.*, 2003). MSSA became methicillin resistant through acquisition of the SCCmec element, probably from other strains, and this occurred on multiple occasions (Robinson and Enright, 2004).

**Figure 1** 
*mecA* gene located SCCmec which encodes for penicillin-binding protein 2a (PBP-2a) and allows *S. aureus* to be methicillin / oxacillin resistant. Expression of PBP-2a controlled by *mecR1* & *mecI* regulator genes located upstream of *mecA* gene. Mutations in the *mec* regulators may phenotypically be highly resistant to methicillin.

**TYPING OF MRSA STRAINS**

MRSA typing systems are important for investigation of outbreaks of infection, aid the clinical treatment of patient, to discriminate between successive and recurrent infections, understanding epidemiology of infections. MRSA strains can be identified/typed using phenotypic and molecular methods.

**Phenotypic typing methods**

Using of colonial characteristics, biochemical reactions, antibiotic susceptibility pattern and susceptibility to various phages.

**Molecular typing methods**

Chromosomal DNA analysis after REA, Pulsed field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), SCCmec typing and spa typing.

**a) Chromosomal DNA analysis after REA**

Chromosomal DNA is subjected to digestion by restriction endonucleases and the resulting fragments are separated by conventional agarose gel electrophoresis. *BglII* and *EcoRI* used for MRSA, as they act on frequently encountered sites along the chromosome, producing short and multiple fragments (Jordens and Hall, 1988). After electrophoresis, set of profiles are compared to those of other isolates. All MRSA isolates are typeable by this method. Restriction profile consists of numerous overlapping fragments, which makes consistent analysis of results difficult (Weller, 2000).

**b) Pulsed field gel electrophoresis (PFGE)**

PFGE used in conjunction with restriction enzymes to provide a DNA fingerprint of the bacterial genome.

**Advantage**

Provides great discrimination between strains and useful in the investigation of outbreaks by allowing differentiation of unrelated strains.

**Disadvantage**

Difficulties with inter-laboratory comparison of results. Reliable comparison of strains between regions and internationally is not always possible.

**c) MLST and SCCmec typing**

Investigation for genetic relatedness of MRSA isolates in international epidemiological studies carried out using results of MLST and SCCmec typing.

**MLST typing**

MLST is an excellent tool for investigating the clonal evolution of MRSA. MLST is based on sequence analysis of 0.5-kb fragments from seven *S. aureus* housekeeping genes, i.e., *arcC*, *aroE*, *gltF*, *gmk*, *pta*, *tpi* and *yqiL* (Maiden *et al.*, 1998). Sequences of the genes compared to known alleles via the MLST website ([http://www.mlst.net](http://www.mlst.net)). Every isolate can be described with seven integer profile. MLST scheme for *S. aureus* was developed in 2000 and details of more than 1500 isolates are available at the *S. aureus* MLST website [http://saureus.mlst.net](http://saureus.mlst.net) (Enright, 2006).

**SCCmec typing**

*mecA* gene is part of larger genetic element known as SCCmec. Element contains the *mecA* gene, chromosomal cassette recombination gene, mec regulatory gene and junkyard region which contains non-essential components of SCCmec. Five SCCmec types currently described which have been characterized using PCR-based techniques. Each type is differentiated by the class of the *mec* gene and the type of recombination genes (Ito *et al.*, 2001, 2004).

**d) spa typing**

Polymorphic DNA sequence analysis of the variation in X region of the protein A gene. This region consists of a number of mainly 24-bp repeats, with its diversity being
attributed mainly to deletions and duplications of the repeats and, more rarely, to point mutations. spa typing of limited number of animal isolates has been carried out. (Voss et al., 2005; Moodley et al., 2006; Strommenger et al., 2006) spa typing differentiates strains that are indistinguishable using PFGE (Moodley et al., 2006).

6. PUBLIC HEALTH SIGNIFICANCE
Several reports suggest that animals may serve as reservoirs for MRSA infection of humans. A cat was implicated as the source of MRSA for nurses in a geriatric nursing facility (Scott et al., 1988). Dog was implicated as a reservoir for the reinfection of two nurses, after their treatment to eliminate carriage of MRSA (Cefai et al., 1994). First case of human to dog transmission was recorded in Netherlands. A nurse was identified as carrier, then treated and tested negative, few week later when she again colonized, family members, environment and pet dog were tested. Pet dog and her daughter were found positive with identical strain (Van Duijkeren et al., 2004).

Recurrence of MRSA infection and nasal colonization in the MRSA infected patients was only halted following successful eradication of MRSA from their family dogs which acted as source of infection for them (Manian, 2003). MRSA isolates from pets in the UK, Ireland and Germany were similar to human isolates. (Baptiste et al., 2005; Loeffler et al., 2005; O’Mahony et al., 2005; Strommenger et al., 2006) In USA, survey of MRSA carriage in small animal veterinary practitioners found similar strain detected in small animals (Hanselman et al., 2006). Malik et al. (2006) compared MRSA human isolates and isolates from dogs and cats and suggested that transmission of MRSA occurs between humans and companion animals and vice versa. Epidemiological typing of equine isolates of MRSA and MRSA strains from equine-associated personnel shows that these isolates were distinct from MRSA strains which commonly cause infections in humans (Baptiste et al., 2005; O’Mahony et al., 2005; Weese et al., 2005; Cuny et al., 2006). Typing data on isolates from pigs and associated human infections and on isolates from cattle suggest MRSA from these species may represent different lineages from those present in humans (Voss et al., 2005; Kwon et al., 2006; Van Dijke et al., 2006). The first case direct transmission of MRSA between cows to humans was documented in Hungary from milk samples (Juhasz-jaszanyitzky et al., 2007).

7. CONTROL OF MRSA IN ANIMALS
Numerous documents on the control of MRSA in people have been published (e.g. CDC MRSA guidelines, available at www.cdc.gov/ncidod/dhap/ar_mrsa.html).

Summary of control measures for MRSA infection and colonization in animals

I) Prevent introduction of infection
Screen all incoming cases for infection and/or nasal carriage at admission to veterinary hospital and isolate until negative status established. It may be possible in referral practices but only screening of suspect cases is practical in first opinion clinics.

II) Prevent transmission from animal-to-human and human-to-animal
Hand hygiene and related measures like Correct hand washing, Use alcohol-based hand sanitizers, Cover wounds and skin lesions, Use gloves, masks, eye protection, disposable aprons for contact with wounds, body fluids or other contaminated materials, Strict asepsis during surgery and Screening staff for MRSA colonization. Based on findings in human medicine, human hands are highly significant for MRSA transmission.

III) Prevent transmission from animal to animal
Isolation of all suspect cases of MRSA infection, No entry to waiting room and Hospitalize in isolation area as far as possible.

IV) Prevent indirect transmission
High standards of cleaning of Hand touch surfaces including doors, pens, stethoscopes, keyboards and High standards of disinfection of consulting rooms/isolation kennels/stables areas following boarding of known animals. In indirect transmission, environmental transmission may be of greater importance than in human medicine.

8. CONCLUSIONS
MRSA first emerged as serious pathogen in human medicine during the late 1970s and has been increasingly reported in animals during the past 10 years. MRSA can be identified using phenotypic (antimicrobial susceptibility testing) or genotypic methods. Epidemiological evidence, including phenotypic and molecular typing data, suggests that MRSA isolates from dogs and cats are indistinguishable from human isolates. Strains of MRSA isolated from horses and associated personnel are found different so far. Transfer of MRSA strains can occur between animals and humans and vice versa. Guidelines for the control of MRSA in animals have been drawn up by individual institutions based on those available for human MRSA infection. Risk factors for MRSA infection in animals are currently under investigation and such data are essential for the preparation of specific guidelines for control of MRSA in veterinary practice.

9. FUTURE PROSPECTS
There is a need for adequate policy framework on infection control that will reflect the current realities on the epidemiologic characters of MRSA. Strict implementation of control program to checkmate the spread of MRSA infections is very much necessary. Epidemiological studies on the spread of MRSA infections at the local, national and international levels will help in designing strategies for preventing the spread.

REFERENCES


by restriction endonuclease digestion of chromosomal DNA. J. Med. Microbiol. 27, 17-123.


